	L #	Hits	Search Text	DBs	Time Stamp
1	L1	2920	BACILLUS ADJ THURINGIENSIS	USPAT; US-PGPUB	2003/04/16 13:03
2	L2	82	CRYVI$2 OR CRY6$2	USPAT; US-PGPUB	2003/04/16 13:04
3	L3	2410	(TRUNCAT$6 OR DIGEST$6 OR FRAGMENT$6) NEAR3 (TOXIN$1 OR (CRYSTAL ADJ PROTEIN$1))	USPAT; US-PGPUB	2003/04/16 13:05
4	L4	51	86A1 OR PS86A1	USPAT; US-PGPUB	2003/04/16 13:06
5	L5	123	1 same 3	USPAT; US-PGPUB	2003/04/16 13:06
6	L6	52	(2 or 4) and 3	USPAT; US-PGPUB	2003/04/16 13:14
7	L7	156	5 or 6	USPAT; US-PGPUB	2003/04/16 13:14

	Document ID	Source	Issue Date	Title
1	US 20030068335	US-PGPUB	20030410	Polynucleotide compositions toxic to diabrotica insects, and methods of use
2	US 20030054391 A1	US-PGPUB	20030320	Formicidae (ant) control using Bacillus thuringiensis toxin
3	US 20030017571 A1	US-PGPUB	20030123	Polynucleotide compositions encoding broad spectrum delta-endotoxins
4	US 20020147148	US-PGPUB	20021010	Insecticidal protein toxins from xenorhabdus
5	US 20020106768	US-PGPUB	20020808	Bacillus thuringiensis toxins with improved activity
6	US 20020100080 A1	US-PGPUB	20020725	Novel pesticidal toxins and nucleotide sequences which encode these toxins
7	US 20020064865 A1	US-PGPUB	20020530	Polynucleotide compositions encoding broad spectrum delta-endotoxins
8	US 20010026940	US-PGPUB	20011004	Plant-optimized genes encoding pesticidal toxins
9	US 20010026939	US-PGPUB	20011004	Insecticidal cotton plant cells
10	US 20010014469 A1	US-PGPUB	20010816	Novel hybrid pesticidal toxins
11	US 20010010932	US-PGPUB	20010802	Nematicidal proteins
12	US 20010001710	US-PGPUB	20010524	Bacillus thuringiensis isolates active against weevils
13	US 6548291 B1	USPAT	20030415	Pesticidal toxins
14	US 6538109 B2	USPAT	20030325	Polynucleotide compositions encoding broad spectrum delta-endotoxins

	Document ID	Source	Issue Date	Title
15	US 6537756 B1	USPAT	20030325	Bacillus thuringiensis CryET29 compositions toxic to coleopteran insects and Ctenocephalides SPP
16	US 6528484 B1	USPAT	20030304	Insecticidal protein toxins from Photorhabdus
17	US 6521442 B2	USPAT	20030218	Polynucleotide compositions encoding broad spectrum .delta.-endotoxins
18	US 6489543 B1	USPAT	20021203	Spring canola (Brassica napus) variety SVO95-08
19	US 6468523 B1	USPAT	20021022	Polypeptide compositions toxic to diabrotic insects, and methods of use
20	US 6455763 B1	USPAT	20020924	Spring canola (Brassica napus) variety "S010"
21	US 6444879 B1	USPAT	20020903	Spring canola (Brassica napus) variety "1709"
22	US 6433254 B1	USPAT	20020813	Spring canola (Brassica napus) variety "Nex 705"
23	US 6423828 B1	USPAT	20020723	Nuclei acid and polypeptide compositions encoding lepidopteran-toxic polypeptides
24	US 6399861 B1	USPAT	20020604	Methods and compositions for the production of stably transformed, fertile monocot plants and cells thereof
25	US 6395966 B1	USPAT	20020528	Fertile transgenic maize plants containing a gene encoding the pat protein
26	US 6379946 B1	USPAT	20020430	Insecticidal protein toxins from Xenorhabdus

	Document ID	Source	Issue Date	Title
27	US 6372480 B1	USPAT	20020416	Pesticidal proteins
28	US 6338846 B1	USPAT	20020115	Recombinant baculovirus, construction method thereof and insect pesticidal composition containing the same
29	US 6329574 B1	USPAT	20011211	High lysine fertile transgenic corn plants
30	US 6326527 B1	USPAT	20011204	Method for altering the nutritional content of plant seed
31	US 6326169 B1	USPAT	20011204	Polynucleotide compositions encoding Cry1Ac/Cry1F chimeric O-endotoxins
32	US 6313378 B1	USPAT	20011106	Lepidopteran-resistent transgenic plants
33	US 6310035 B1	USPAT	20011030	Polypeptides endowed with a larvicidal activity toward Lepidoptera
34	US 6303364 B1	USPAT	20011016	Bacillus thuringiensis toxins with improved activity
35	US 6294184 B1	USPAT	20010925	Process for controlling lepidopteron pests
36	US 6291156 B1	USPAT	20010918	Plant pest control
37	US 6281411 B1	USPAT	20010828	Transgenic monocots plants with increased glycine-betaine content

	Document ID	Source	Issue Date	Title
38	US 6281016 B1	USPAT	20010828	Broad-spectrum insect resistant transgenic plants
39	US 6271016 B1	USPAT	20010807	Anthranilate synthase gene and method of use thereof for conferring tryptophan overproduction
40	US 6242669 B1	USPAT	20010605	Pesticidal toxins and nucleotide sequences which encode these toxins
41	US 6242241 B1	USPAT	20010605	Polynucleotide compositions encoding broad-spectrum .delta.-endotoxins
42	US 6221649 B1	USPAT	20010424	Chimeric bacillus thuringiensis-endotoxins and host cells expressing same
43	US 6218188 B1	USPAT	20010417	Plant-optimized genes encoding pesticidal toxins

	Document ID	Source	Issue Date	Title
44	US 6204435 B1	USPAT	20010320	Pesticidal toxins and nucleotide sequences which encode these toxins
45	US 6204246 B1	USPAT	20010320	Hybrid toxin
46	US 6177615 B1	USPAT	20010123	Lepidopteran-toxic polypeptide and polynucleotide compositions and methods for making and using same
47	US 6166195 A	USPAT	20001226	Nematode-active toxins and genes which code therefor
48	US 6160208 A	USPAT	20001212	Fertile transgenic corn plants
49	US 6156573 A	USPAT	20001205	Hybrid Bacillus thuringiensis .delta.-endotoxins with novel broad-spectrum insecticidal activity
50	US 6153814 A	USPAT	20001128	Polypeptide compositions toxic to lepidopteran insects and methods for making same

	Document ID	Source	Issue Date	Title
51	US 6137033 A	USPAT	20001024	Class of proteins for the control of plant pests
52	US 6127180 A	USPAT	20001003	Pesticidal toxins
53	US 6118047 A	USPAT	20000912	Anthranilate synthase gene and method of use thereof for conferring tryptophan overproduction
54	US 6110734 A	USPAT	20000829	Nucleotide sequences coding for polypeptides endowed with a larvicidal activity towards lepidoptera
55	US 6110464 A	USPAT	20000829	Broad-spectrum .delta.-endotoxins
56	US 6107546 A	USPAT	20000822	Transformation vectors allowing expression of truncated BT2 endotoxins in plants
57	US 6083499 A	USPAT	20000704	Pesticidal toxins
58	US 6071511 A	USPAT	20000606	Bacillus thuringiensis isolates, toxins, and genes selectively active against certain coleopteran pests
59	US 6063597 A	USPAT	20000516	Polypeptide compositions toxic to coleopteran insects
60	US 6060594 A	USPAT	20000509	Nucleic acid segments encoding modified bacillus thuringiensis coleopteran-toxic crystal

	Document ID	Source	Issue Date	Title
61	US 6051556 A	USPAT	20000418	Hybrid pesticidal toxins
62	US 6051550 A	USPAT	20000418	Materials and methods for controlling homopteran pests
63	US 6048839 A	USPAT	20000411	Materials and methods for controlling insect pests with pesticidal proteins obtainable from Bacillus thuringiensis isolates PS158C2 and
64	US 6048838 A	USPAT	20000411	Insecticidal protein toxins from xenorhabdus
65	US 6043415 A	USPAT	20000328	Synthetic Bacillus thuringiensis cryic gene encoding insect toxin
66	US 6040504 A	USPAT	20000321	Cotton promoter
67	US 6037527 A	USPAT	20000314	Expression of proteins in plants using an AMV coat protein leader sequence
68	US 6033874 A	USPAT	20000307	CRY1C polypeptides having improved toxicity to lepidopteran insects
69	US 6028246 A	USPAT	20000222	Bacillus thuringiensis strains and their insecticidal proteins
70	US 6025545 A	USPAT	20000215	Methods and compositions for the production of stably transformed, fertile monocot plants and cells thereof

	Document ID	Source	Issue Date	Title
71	US 6023013 A	USPAT	20000208	Insect-resistant transgenic plants
72	US 6017534 A	USPAT	20000125	Hybrid Bacillus thuringiensis .delta.-endotoxins with novel broad-spectrum insecticidal activity
73	US 6013863 A	USPAT	20000111	Fertile transgenic corn plants
74	US 5990390 A	USPAT	19991123	Methods and compositions for the production of stably transformed, fertile monocot plants and cells thereof
75	US 5985831 A	USPAT	19991116	Methods for controlling lepidopterans using Bacillus thuringiensis toxins obtainable from isolates PS17, PS86Q3, and HD511
76	US 5981698 A	USPAT	19991109	Antimicrobial polypeptides
77	US 5977058 A	USPAT	19991102	Antiproliferative protein from Bacillus thuringiensis var. thuringiensis
78	US 5969213 A	USPAT	19991019	Methods and compositions for the production of stably transformed fertile monocot plants and cells thereof
79	US 5959091 A	USPAT	19990928	Truncated gene of Bacillus thuringiensis encoding a polypeptide toxin
80	US 5942664 A	USPAT	19990824	Bacillus thuringiensis Cry1C compositions toxic to lepidopteran insects and methods for making Cry1C mutants
81	US 5932209 A	USPAT	19990803	Bacillus thuringiensis .delta.-endotoxin

	Document ID	Source	Issue Date	Title
82	US 5928891 A	USPAT	19990727	DNA fragment encoding insecticidal crystal proteins from Bacillus thuringiensis
83	US 5914318 A	USPAT	19990622	Transgenic plants expressing lepidopteran-active .delta.-endotoxins
84	US 5877012 A	USPAT	19990302	Class of proteins for the control of plant pests
85	US 5874289 A	USPAT	19990223	Bacillus thuringiensis mutants which produce high yields of crystal delta-endotoxin
86	US 5874288 A	USPAT	19990223	Bacillus thuringiensis toxins with improved activity
87	US 5874265 A	USPAT	19990223	Methods and compositions for the production of stably transformed fertile monocot plants and cells thereof
88	US 5854053 A	USPAT	19981229	Bacillus thuringiensis bacteria
89	US 5843898 A	USPAT	19981201	Transformation vectors allowing expression of foreign polypeptide endotoxins in plants
90	US 5840554 A	USPAT	19981124	.beta.-Endotoxin expression in pseudomonas fluorescens

	Document ID	Source	Issue Date	Title
91	US 5837237 A	USPAT	19981117	Bacillus thuringiensis strains and their genes encoding insecticidal toxins
92	US 5831011 A	USPAT	19981103	Bacillus thuringiensis genes encoding nematode-active toxins
93	US 5827514 A	USPAT	19981027	Pesticidal compositions
94	US 5824636 A	USPAT	19981020	Antiproliferative protein from Bacillus thuringiensis var. thuringiensis
95	US 5824302 A	USPAT	19981020	Method of controlling insect larvae comprising feeding an insecticidal amount of a transgenic maize plant expressing a polypeptide having Bt-crystal protein toxic properties
96	US 5792928 A	USPAT	19980811	Nucleotide sequences coding for polypeptides endowed with a larvicidal activity towards lepidoptera
97	US 5780709 A	USPAT	19980714	Transgenic maize with increased mannitol content
98	US 5770450 A	USPAT	19980623	Zea mays plants regenerated from protoplasts or protoplast-derived cells
99	US 5767372 A	USPAT	19980616	Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus thuringiensis in plants
100	US 5766900 A	USPAT	19980616	Method of regenerating fertile transgenic Zea mays plants from protoplasts
101	US 5760181 A	USPAT	19980602	Endotoxins

	Document ID	Source	Issue Date	Title
102	US 5736131 A	USPAT	19980407	Hybrid toxin
103	US 5731194 A	USPAT	19980324	Insecticide protein and gene
104	US 5723756 A	USPAT	19980303	Bacillus thuringiensis strains and their genes encoding insecticidal toxins
105	US 5723440 A	USPAT	19980303	Controlling hemipteran insect pests with Bacillus thuringiensis
106	US 5712248 A	USPAT	19980127	Method of controlling insect with novel insecticidal protein
107	US 5707619 A	USPAT	19980113	Bacillus thuringiensis isolates active against weevils
108	US 5683691 A	USPAT	19971104	Bacillus thuringiensis insecticidal toxins
109	US 5679343 A	USPAT	19971021	Bacillus thuringiensis cryET4 and cryET5 protein insecticidal composition and method of use
110	US 5670365 A	USPAT	19970923	Identification of, and uses for, nematicidal bacillus thuringiensis genes, toxins, and isolates
111	US 5659123 A	USPAT	19970819	Diabrotica toxins

	Document ID	Source	Issue Date	Title
112	US 5658781 A	USPAT	19970819	Insecticidally effective peptides
113	US 5658563 A	USPAT	19970819	Insecticidally effective peptides
114	US 5640804 A	USPAT	19970624	Pest trap plants and crop protection
115	US 5616319 A	USPAT	19970401	Bacillus thuringiensis cryET5 gene and related plasmids, bacteria and insecticides
116	US 5608142 A	USPAT	19970304	Insecticidal cotton plants
117	US 5595733 A	USPAT	19970121	Methods for protecting ZEA mays plants against pest damage
118	US 5593881 A	USPAT	19970114	Bacillus thuringiensis delta-endotoxin
119	US 5578702 A	USPAT	19961126	Toxin active against lepidopteran insects
120	US 5554798 A	USPAT	19960910	Fertile glyphosate-resistant transgenic corn plant
121	US 5545818 A	USPAT	19960813	Expression of Bacillus thuringiensis cry proteins in plant plastids
122	US 5545817 A	USPAT	19960813	Enhanced expression in a plant plastid

	Document ID	Source	Issue Date	Title
123	US 5545565 A	USPAT	19960813	Transformation vectors allowing expression of foreign polypeptide endoxins from Bacillus thuringiensis in plants
124	US 5530195 A	USPAT	19960625	Bacillus thuringiensis gene encoding a toxin active against insects
125	US 5508264 A	USPAT	19960416	Pesticidal compositions
126	US 5508032 A	USPAT	19960416	Bacillus thuringiensis isolates active against cockroaches
127	US 5466597 A	USPAT	19951114	Bacillus thuringiensis strains and their genes encoding insecticidal toxins
128	US 5461032 A	USPAT	19951024	Insecticidally effective peptides
129	US 5356623 A	USPAT	19941018	Bacillus thuringiensis cryET1 toxin gene and protein toxic to lepidopteran insects
130	US 5350689 A	USPAT	19940927	Zea mays plants and transgenic Zea mays plants regenerated from protoplasts or protoplast-derived cells
131	US 5349124 A	USPAT	19940920	Insect-resistant lettuce plants
132	US 5322687 A	USPAT	19940621	Bacillus thuringiensis cryet4 and cryet5 toxin genes and proteins toxic to lepidopteran insects
133	US 5317096 A	USPAT	19940531	Transformation vectors allowing expression of foreign polypeptide endotoxins from Bacillus thuringiensis in plants
134	US 5306628 A	USPAT	19940426	Method and means for extending the host range of insecticidal proteins
135	US 5290914 A	USPAT	19940301	Hybrid diphtheria-B.t. pesticidal toxins

	Document ID	Source	Issue Date	Title
136	US 5281532 A	USPAT	19940125	Pseudomas hosts transformed with bacillus endotoxin genes
137	US 5273746 A	USPAT	19931228	Bacillus thuringiensis isolates active against phthiraptera pests
138	US 5262159 A	USPAT	19931116	Use of Bacillus thuringiensis isolates for controlling pests in the family aphididae
139	US 5262158 A	USPAT	19931116	Bacillus thuringiensis isolates for controlling acarida
140	US 5254799 A	USPAT	19931019	Transformation vectors allowing expression of Bacillus thuringiensis endotoxins in plants
141	US 5229112 A	USPAT	19930720	Combatting plant insect pests with plant-colonizing microorganisms containing the toxin gene B. thuringiensis as a chromosomal
142	US 5143905 A	USPAT	19920901	Method and means for extending the host range of insecticidal proteins
143	US 5110905 A	USPAT	19920505	Activated Bacillus thuringienses delta-endotoxin produced by an engineered hybrid gene
144	US 5055294 A	USPAT	19911008	Chimeric Bacillus thuringiensis crystal protein gene comprising HD-73 and Berliner 1715 toxin genes, transformed and expressed in Pseudomonas fluorescens
145	US 5010001 A	USPAT	19910423	Preparation of natural or modified insect toxins
146	US H000875 H	USPAT	19910101	Toxin-encoding nucleic acid fragments derived from a Bacillus thuringiensis subsp. israelensis gene

	Document ID	Source	Issue Date	Title
147	US 4695455 A	USPAT	19870922	Cellular encapsulation of pesticides produced by expression of heterologous genes
148	US 4467036 A	USPAT	19840821	Bacillus thuringiensis crystal protein in Escherichia coli

US-PAT-NO: **6344553**

DOCUMENT-IDENTIFIER: US 6344553 B1

TITLE: Bacillus thuringiensis toxins and genes for controlling coleopteran pests

DATE-ISSUED: February 5, 2002

INVENTOR-INFORMATION:

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APPL-NO: 09/ 307925

DATE FILED: May 10, 1999

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This application is a continuation-in-part of application Ser. No. 09/076,193, filed May 12, 1998 issued as U.S. Pat. No. 5,973,231.

US-CL-CURRENT: 536/23.71, 435/252.3 , 435/419

ABSTRACT:

The subject invention concerns materials and methods useful in the control of pests and, particularly, the plant pests. More specifically, the subject invention concerns novel genes and pesticidal toxins referred to as **86A1**(b) and 52A1(b). In preferred embodiments, the subject toxins are used for controlling flea beetles of the genus Phyllotreta. Using the genes described herein, the transformation of plants can be accomplished using techniques known to those skilled in the art. In addition, the subject invention provides toxin genes optimized for expression in plants.

26 Claims, 0 Drawing figures

Exemplary Claim Number: 1

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Abstract Text - ABTX (1):

The subject invention concerns materials and methods useful in the control of pests and, particularly, the plant pests. More specifically, the subject invention concerns novel genes and pesticidal toxins referred to as **86A1**(b) and 52A1(b). In preferred embodiments, the subject toxins are used for controlling flea beetles of the genus Phyllotreta. Using the genes described herein, the transformation of plants can be accomplished using techniques known to those skilled in the art. In addition, the subject invention provides toxin genes optimized for expression in plants.

US Patent No. - PN (1):

6344553

Brief Summary Text - BSTX (13):

Hofte and Whiteley (Hofte, H., H. R. Whiteley [1989] Microbiological Reviews 52(2):242-255) classified B.t. crystal protein genes into four major classes. The classes were CryI (Lepidoptera-specific),CryII (Lepidoptera- and Diptera-specific), CryIII (Coleoptera-specific), and CryIV (Diptera-specific). CryV and **CryVI** were proposed to designate a class of toxin genes that are nematode-specific. Other classes of B.t. genes have now been identified.

Brief Summary Text - BSTX (15):

B.t. isolate **PS86A1** is disclosed in the following U.S. Pat. No. 4,849,217 (activity against alfalfa weevil); U.S. Pat. No. 5,208,017 (activity against corn rootworm); U.S. Pat. No. 5,286,485 (activity against lepidopterans); and U.S. Pat. No. 5,427,786 (activity against Phyllotreta genera). A gene from **PS86A1** was cloned into B.t. MR506, which is disclosed in U.S. Pat. No. 5,670,365 (activity against nematodes) and PCT international patent application publication No. WO93/04587 (activity against lepidopterans). The sequences of a gene and a **Cry6A (CryVIA)** toxin from **PS86A1** are disclosed in the following U.S. Pat. No. 5,186,934 (activity against Hypera genera); U.S. Pat. No. 5,273,746 (lice); U.S. Pat. Nos. 5,262,158 and 5,424,410 (activity against mites); as well as in PCT international patent application publication No. WO94/23036 (activity against wireworms). U.S. Pat. Nos. 5,262,159 and 5,468,636, disclose **PS86A1,** the sequence of a gene and toxin therefrom, and a generic formula for toxins having activity against aphids.

Brief Summary Text - BSTX (16):

B.t. isolate PS52A1 is disclosed by the following U.S. patents as being active against nematodes: U.S. Pat. Nos. 4,861,595; 4,948,734, 5,093,120, 5,262,399, 5,236,843, 5,322,932; and 5,670,365. PS52A1 is also disclosed in U.S. Pat. No. 4,849,217,supra, and PCT international patent application publication No. WO95/02694 (activity against Calliphoridae). The sequences of a gene and a nematode-active toxin from PS52A1 are disclosed in U.S. Pat. No. 5,439,881 and European patent application publication No. EP 0462721. PS52A1,

the sequence of a gene and nematode-activetoxin therefrom, and a generic formula for **CryVIA** toxins are disclosed in PCT international patent application publication No. WO 92/19739.

Brief Summary Text - BSTX (18):

Although B.t. strains **PS86A1** and PS52A1, and a gene and toxin therefrom, were known to have certain pesticidal activity, additional genes encoding active toxins from these isolates were not previously known in the art.

Brief Summary Text - BSTX (20):

The subject invention provides novel genes encoding pesticidal toxins. Preferred, novel toxin genes of the subject invention are designated **86A1**(b) and 52A1(b). These genes encode toxins that are active against plant pests, preferably insects, preferably coleopterans, and most preferably flea beetles of the genus Phyllotreta.

Brief Summary Text - BSTX (25):

SEQ ID NO. 1 is a forward oligonucleotide probe for 52A1(b) and **86A1**(b).

Brief Summary Text - BSTX (26):

SEQ ID NO. 2 is a nucleotide sequence of a gene encoding the **86A1** (b) toxin.

Brief Summary Text - BSTX (27):

SEQ ID NO. 3 is an amino acid sequence of the **86A1** (b) toxin.

Brief Summary Text - BSTX (32):

SEQ ID NO. 8 is a preferred, truncated version of the full-length, native 52A1(b) toxin. In the gene encoding this toxin (and for the genes encoding all of the following amino acid sequences shown in SEQ ID NOS. 9-19), the initiator codon for methionine has been added so that the N-terminal amino acid is methionine and not leucine (leucine is the first amino acid in the native protein). This truncation and the proteins shown in SEQ ID NOS. 9-13 have N-terminal deletions from the native protein. The natural 52A1(b) end is otherwise used in these truncations. After the first amino acid, this **truncated toxin** begins with amino acid 10 of the native protein. That is, the first 9 amino acids of the native protein have been replaced in favor of the single amino acid methionine. The remaining (C-terminal) portion of this toxin is the same as that of the native protein. In preferred embodiments, two stop codons are used in the gene encoding this toxin as well as in the genes encoding the following truncated proteins (SEQ ID NOS. 9-19).

Brief Summary Text - BSTX (45):

The subject invention provides novel genes encoding pesticidal toxins. Preferred, novel toxin genes of the subject invention are designated **86A1**(b) and 52A1(b). These genes encode toxins that are active against (which can be used to control, or which are toxic to, or which are lethal to) plant pests, preferably insects, preferably coleopterans, and most preferably flea beetles of the genus Phyllotreta. The use of the subject genes and toxins for controlling other pests, such as pests of the genus Psylliodes, is also contemplated.

Brief Summary Text - BSTX (48):

Characteristics of Bacillus thuringiensis isolates **PS86A1** and PS52A1, such as colony morphology, inclusiontype, and the sizes of alkali-solubleproteins (by SDS-PAGE), have been disclosed in, for example, U.S. Pat. No. 5,427,786 and published PCT application WO 95/02694, respectively.

Brief Summary Text - BSTX (54):

Fragments of the genes and toxins specifically exemplified herein which retain the pesticidal activity of the exemplified toxins are within the scope of the subject invention. Genes and toxins useful according to the subject invention include not only the full length sequences but also fragments of these sequences which retain the characteristic pesticidal activity of the toxins specifically exemplified herein.

Brief Summary Text - BSTX (56):

Genes can be modified, and variations of genes may be readily constructed, as would be known to one skilled in the art. For example, U.S. Pat. No. 5,605,793 describes methods for generating additional molecular diversity by using DNA reassembly after random fragmentation. Standard techniques are available for making point mutations. The use of site-directed mutagenesis is known in the art. Fragments of the subject genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or can be used to systematically cut off nucleotides from the ends of these genes. Useful genes may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active **fragments of these toxins**.

Brief Summary Text - BSTX (60):

There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other Bacillus toxins. These

antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent **t xins or fragments f these toxins,** can readily be prepared using standard procedures in this art.

Brief Summary Text - BSTX (64):

Full length B.t. toxins can be expressed and then converted to active, truncated forms through the addition of appropriate reagents and/or by growing the cultures under conditions which result in the truncation of the proteins through the fortuitous action of endogenous proteases. In an alternative embodiment, the full length toxin may undergo other modifications to yield the active form of the toxin. Adjustment of the solubilization of the toxin, as well as other reaction conditions, such as pH, ionic strength, or redox potential, can be used to effect the desired modification of the toxin. **Truncated toxins** of the subject invention can be obtained by treating the crystalline .delta.-endotoxin of **Bacillus thuringiensis** with a serine protease such as bovine trypsin at an alkaline pH and preferably in the absence of .beta.-mercaptoethanol.

Brief Summary Paragraph Table - BSTL (1):

TABLE 1 Repository Culture Accession No. Deposit date B.t. var. wuhanensis **PS86A1** NRRL B-18400 August 16, 1988 B.t. var. wuhanensis PS52A1 NRRL B-18245 July 28, 1987

Detailed Description Text - DETX (9):

Molecular Cloning, Expression, and Sequencing of Novel Toxin Genes From Bacillus thuringiensis Strains PS52A1 and **PS86A1**

Detailed Description Text - DETX (10):

Total cellular DNA was prepared from PS52A1 and **PS86A1** Bacillus thuringiensis (B.t.) cells grown at 30.degree. C. to an optical density of 1.0 at 600 nm. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/mL lysozyme in 0.3M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation at 37.degree. C. for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1 M NaCl, 0.1% SDS, 0.1 M Tris-Cl [pH 8.0] were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in TE buffer (10 mM Tris-Cl [pH 8.0], 1 mM EDTA) and RNase was added to a final concentration of 50 .mu.g/mL. After incubation at 37.degree. C. for 1 hour, the solution was extracted once each with phenol:chloroform(1:1) and TE-saturatedchloroform. From the aqueous phase, DNA was precipitated by the addition of one-tenth volume 3M NaOAc and two volumes ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried,

and resuspended in TE buffer.

Detailed Description Text - DETX (11):

Plasmid DNA was also prepared from B.t. strain **PS86A1**. The B.t. cells were grown at 30.degree. C. to an optical density of 1.0 at 600 nm. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/mL lysozyme in 0.3M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation on ice for 30 minutes, ten volumes of lysis buffer (0.085 M NaOH, 0.1% SDS in TE buffer)were added. The lysate was rocked gently at room temperature for 30 minutes. One-half volume 3M KOAc was added to the suspension for incubation at 4.degree. C. overnight. Nucleic acids were precipitated with one volume isopropanol and pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer. The DNA suspension was further purified by extraction once with phenol:chloroform (1:1). DNA in the aqueous phase was precipitated by the addition of one-tenth volume 3M NaOAc and one volume of isopropanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer. CsCl was added at equal weight to volume of DNA solution, and ethidium bromide was added to a final concentration of 0.5 mg/mL. The plasmid DNA was separated from the extraneous nucleic acids by overnight ultracentrifugation. The recovered plasmid band was extracted five times with excess water-saturated butanol, and dialyzed against TE buffer. DNA was precipitated, pelleted, washed, dried and resuspended in TE buffer as described previously. Based on N-terminal amino acid sequencing data of the **PS86A1** 45 kDa polypeptide, the following "forward" oligonucleotide of sequence (SEQ ID NO. 1) was synthesized for use in Southern hybridizations:

Detailed Description Text - DETX (13):

PS86A1 total cellular and plasmid DNA were digested with selected restriction endonucleases, electrophoresed on an agarose gel, subsequently blotted onto a nylon membrane, and immobilized by "baking" the membrane at 80.degree. C. Restriction fragment length polymorphism (RFLP) analysis was performed using the oligonucleotide probe described above. Southern blots were hybridized overnight in 6.times. SSPE, 5.times. Denhardt's solution, 0.1 mg/mL single stranded carrier DNA and 0.1% SDS at 37.degree. C. The blots were then washed in 1.times. SSPE, 0.1% SDS at 37.degree. C., air-dried, then exposed to X-ray film. Autoradiography identified an approximately 6.6 kbp Xba I band in both the total cellular and plasmid DNA blots that was theorized to contain all or part of the PS86B 1(b) toxin gene.

Detailed Description Text - DETX (14):

The approximately 6.6 kbp Xba I fragment was cloned into pHTBluell (an E. coli/B. thuringiensis shuttle vector composed of pBluescript II SK--(Stratagene, La Jolla, Calif.) and the replication origin from a resident B.t. plasmid Lereclus et al. [1989] FEMS Microbiology Letters 60:211-218]). Polymerase chain reaction (PCR) mapping to determine if the fragment contained the full-length gene was conducted using the "forward" oligonucleotide primer described previously and vector primers. The "forward" primer combined with

vector primer T7 resulted in amplification of only an approximately 400 bp-sized fragment, instead of the approximately 1.0 kbp gene expected to encode a protein of 45 kDa length. This established that only approximately one-third of the **PS86A1**(b) toxin gene was cloned. Further verification was provided by dideoxynucleotide sequencing (Sanger et al. [1977] Proc. Natl. Acad. Sci. USA 74:5463-5467) using Sequenase (US Biochemical, Cleveland, Ohio) on the subgene construct. The PCR fragment was subsequently radiolabelled with .sup.32 P and used as a probe in standard hybridizations of Southern blots and gene libraries of **PS86A1** and PS52A1 total cellular DNA.

Detailed Description Text - DETX (15):

A gene library was constructed from **PS86A1** total cellular DNA partially digested with Sau3A I. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column (Schleicher and Schuell, Keene, N.H.), and recovered by ethanol precipitation. The Sau3A I inserts were ligated into BamHI-digested LambdaGem-11 (Promega, Madison, Wis.). Recombinant phage were packaged and plated on E coil KW251 (Promega, Madison, Wis.) cells. Plaques were screened by transfer of recombinant phage DNA to filters and hybridization with the PCR probe described previously. Hybridization was carried out overnight at 37.degree. C. in a solution consisting of 6.times. SSPE, 5.times. Denhardt's solution, 0.1 mg/mL single stranded carrier DNA, and 0.1% SDS. The filters were subsequently washed in 1.times. SSPE and 0.1% SDS at 37.degree. C., air-dried, and then exposed to X-ray film. Hybridizing phage were plaque-purified and used to infect liquid cultures of E. coli KW251 cells for isolation of DNA by standard procedures (Maniatis et al. [1982] Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Southern blotting of plaque-purifiedhybridizing phage DNA digested with selected restriction endonucleases using the PCR-amplified probe and washing conditions as described above revealed an approximately 2.3 kbp EcoR V+Sal I fragment believed to contain the **PS86A1**(b) gene.

Detailed Description Text - DETX (16):

For subcloning the **PS86A1**(b) gene encoding the approximately 45 kDa toxin, preparative amounts of phage DNA were digested with EcoRV and Sall. The approximately 2.3 kbp band was ligated into Smal+Sall-digested pHTBluell. The ligation mix was used to transform frozen, competent E. coil NM522 cells (ATCC 47000). .beta.-galactosidase-negative transformants were screened by restriction digestion of alkaline lysate plasmid miniprep DNA. The desired plasmid construct, pMYC2344,contains the **PS86A1**(b) toxin gene. pMYC2344 was introduced into the acrystalliferous (Cry-) B.t. host, CryB (A. Aronson, Purdue University, West Lafayette, Ind.) by electroporation. Expression of the toxin was demonstrated by visualization of crystal formation under microscopic examination, and SDS-PAGE analysis. Gene construct pMYC2344 in B.t. is designated MR509.

Detailed Description Text - DETX (17):

A sequence of the **86A1**(b) gene is shown in SEQ ID NO.2. A deduced amino acid sequence for the **86A1**(b) toxin is shown in SEQ ID NO. 3.

Detailed Description Text - DETX (18):

The **PS86A1**(b) probes, hybridization, and washing conditions were also used to clone a related gene, PS52A1(b), from Bacillus thuringiensis strain PS52A1. A gene library was constructed by partially digesting PS52A1 total cellular DNA with Sau3A 1. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column, and recovered by ethanol precipitation. The Sau3A I inserts were ligated into BamHI-digested LambdaGem-11. Recombinant phage were packaged and plated on E. coli KW251 cells. Plaques were screened by hybridization with the PCR probe described previously. Hybridizing phage were plaque-purified and used to infect liquid cultures of E. coli KW251 cells for isolation of DNA by standard procedures. Southern blotting of plaque-purified hybridizing phage DNA digested with selected restriction endonucleases using the PCR probe revealed an approximately 2.3 kbp EcoRV+SalI fragment believed to contain the PS52A1(b) gene.

Detailed Description Text - DETX (22):

Bioassay of the MR509/**86A1**(b) Toxin Against Phyllotreta

Detailed Description Text - DETX (24):

Several treatments showed reduced plant damage relative to untreated and CryB (a crystal-minus B.t. strain) controls. It was determined that the approximately 45 kda protein from MR509 was highly active against the tested Phyllotreta cruciferae pests; this toxin is referred to as the **86A1**(b) gene.

Detailed Description Text - DETX (26):

Further Bioassays: MR509/**86A1**(b) and MR510/52A1(b) Against Phyllotreta spp.

Detailed Description Text - DETX (31):

Truncations of the Native **86A1**(b) and 52A1(b) Toxins

Detailed Description Text - DETX (32):

Using techniques known to those skilled in the art, some of which are discussed above, the native proteins can be truncated. These **truncated toxins** can be screened for activity by one skilled in the art using the guidance provided herein together with what is known in the art. Preferred, truncated

proteins are shown in SEQ ID NOS. 8-19. The subject invention also includes polynucleotides that encode the exemplified, truncated proteins, as well as other truncations, **fragments, and variants of the exemplified toxins, s long as the truncations,** fragments, or variants retain pesticidal activity, preferrably against coleopterans, and most preferably against flea beetles.

Detailed Description Text - DETX (33):

Truncated toxins according to the subject invention include not only toxins having deletions in the N-terminal or C-terminal portions as exemplified herein, but also toxins having deletions to both the N-terminal and C-terminal portions of the native protein. Examples of such truncations would include proteins resulting from using any of the N-terminal deletions exemplified herein together with any of the C-terminal deletions exemplified herein.

Detailed Description Text - DETX (35):

Further Characterization of **86A1**(b) and 52A1(b) Toxins

Detailed Description Text - DETX (36):

A polyclonal antibody referred to as R#56 was developed and purified to the native toxin 52A1(b). This antibody recognizes the native **86A1**(b) toxin. This antibody can be used in blotting screens (dot, slot, and/or western blots) to determine if homologs of the 52A 1(b) and 86A 1(b) toxins are present in other strains of Bacillus.

Detailed Description Text - DETX (37):

Thus, in further embodiment of the subj ect invention, additional pesticidal toxins can be characterized and/or identified by their level of reactivity with antibodies to pesticidal toxins exemplified herein. Antibodies can be raised to the specifically exemplified toxins of the subject invention. Other toxins within the scope of this invention can then be identified and/or characterized by their reactivity with the antibodies. In a preferred embodiment, the antibodies are polyclonal antibodies. In this embodiment, toxins with the greatest similarity to the **86A1**(b) or 52A 1(b) toxins would have the greatest reactivity with the polyclonal antibodies. Toxins with greater diversity react with polyclonal antibodies, but to a lesser extent.

Detailed Description Text - DETX (46):

In a preferred embodiment of the subject invention, plants will be transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Pat. No. 5,380,831. Also, advantageously, DNA encoding a **truncated toxin** will be used. The **truncated toxin** typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic Bacillus genes for use in plants are known in the art.

US-PAT-NO: **6077824**

DOCUMENT-IDENTIFIER: US 6077824 A

TITLE: Methods for improving the activity of .delta.-endotoxins against insect pests

DATE-ISSUED: June 20, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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APPL-NO: 08/ 993775

DATE FILED: December 18, 1997

US-CL-CURRENT: 514/12, 435/69.1 , 514/2 , 530/350 , 530/402

ABSTRACT:

Disclosed are methods for increasing the activity of B. thuringiensis .delta.-endotoxins against Coleopteran insect pests. Also disclosed are methods for mutagenizing nucleic acid sequences encoding these polypeptides, and increasing insect resistance in transgenic plants expressing these genes.

41 Claims, 23 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 19

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US Patent No. - PN (1):

6077824

Brief Summary Text - BSTX (13):

.delta.-endotoxins are used to control a wide range of leaf-eating caterpillars and beetles, as well as mosquitoes. These proteinaceous parasporal crystals, also referred to as insecticidal crystal proteins, crystal proteins, Bt inclusions, crystaline inclusions, inclusion bodies, and Bt toxins, are a large collection of insecticidal proteins produced by B. thuringiensis that are toxic upon ingestion by a susceptible insect host. Over the past decade research on the structure and function of B. thuringiensis toxins has covered all of the major toxin categories, and while these toxins differ in specific structure and function, general similarities in the structure and function are assumed. Based on the accumulated knowledge of B. thuringiensis toxins, a generalized mode of action for B. thuringiensis toxins has been created and includes: ingestion by the insect, solubilization in the insect midgut (a combination stomach and small intestine), resistance to digestive enzymes sometimes with partial **digestion actually "activating" the toxin,** binding to the midgut cells, formation of a pore in the insect cells and the disruption of cellular homeostasis (English and Slatin, 1992).

Brief Summary Paragraph Table - BSTL (1):

TABLE 1 _____ KNOWN B. THURINGIENSIS .delta.-ENDOTOXINS, GENBANK ACCESSION NUMBERS, AND REVISED NOMENCLATURE.sup.A

New Old GenBank Accession # _____

Cry1Aa1 CrylA(a) M11250 Cry1Aa2 CrylA(a) M10917 CryiAa3 CrylA(a) D00348 Cry1Aa4 CrylA(a) X13535 Cry1Aa5 CrylA(a) D17518 Cry1Aa6 CrylA(a) U43605 Cry1Ab1 CrylA(b) M13898 Cry1Ab2 CrylA(b) M12661 Cry1Ab3 CrylA(b) M15271 Cry1Ab4 CrylA(b) D00117 Cry1Ab5 CrylA(b) X04698 Cry1Ab6 CrylA(b) M37263 Cry1Ab7 CrylA(b) X13233 Cry1Ab8 CrylA(b) M16463 Cry1Ab9 CrylA(b) X54939 Cry1Ab10 CrylA(b) Cry1Ac1 CrylA(c) M11068 Cry1Ac2 CrylA(c) M35524 Cry1Ac3 CrylA(c) X54159 Cry1Ac4 CrylA(c) M73249 Cry1Ac5 CrylA(c) M73248 Cry1Ac6 CrylA(c) U43606 Cry1Ac7 CrylA(c) U87793 Cry1Ac8 CrylA(c) U87397 Cry1Ac9 CrylA(c) U89872 Cry1Ac10 CrylA(c) AJ002514 Cry1Ad1 CrylA(d) M73250 Cry1Ae1 CrylA(e) M65252 Cry1Ba1 CrylB X06711 Cry1Ba2 X95704 Cry1Bb1 ET5 L32020 Cry1Bc1 Crylb(c) Z46442 Cry1Bd1 CryE1 Cry1Ca1 CrylC X07518 Cry1Ca2 CrylC X13620 Cry1Ca3 CrylC M73251 Cry1Ca4 CrylC A27642 Cry1Ca5 CrylC X96682 Cry1Ca6 CrylC X96683 Cry1Ca7 CrylC X96684 Cry1Cb1 CrylC(b) M97880 Cry1Da1 CrylD X54160 Cry1Db1 PrtB Z22511 Cry1Ea1 CrylE X53985 Cry1Ea2 CrylE X56144 Cry1Ea3 CrylE M73252 Cry1Ea4 U94323 Cry1Eb1 CrylE(b) M73253 Cry1Fa1 CrylF M63897 Cry1Fa2 CrylF M63897 Cry1Fb1 PrtD Z22512 Cry1Ga1 PrtA Z22510 Cry1Ga2 CrylM Y09326 Cry1Gb1 CryH2 Cry1Ha1 PrtC Z22513 Cry1Hb1 U35780 Cry1Ia1 CryV X62821 Cry1Ia2 CryV M98544 Cry1Ia3 CryV L36338 Cry1Ia4 CryV L49391 Cry1Ia5 CryV Y08920 Cry1Ib1 CryV U07642 Cry1Ja1 ET4 L32019 Cry1Jb1 ET1 U31527 Cry1Ka1 U28801 Cry2Aa1 CrylIA M31738 Cry2Aa2 CrylIA M23723 Cry2Aa3 D86084 Cry2Ab1 CrylIB M23724 Cry2Ab2 CrylIB X55416 Cry2Ac1 CrylIC X57252 Cry3Aa1 CrylIIA M22472 Cry3Aa2 CrylIIA J02978 Cry3Aa3 CrylIIA Y00420 Cry3Aa4 CrylIIA M30503 Cry3Aa5 CrylIIA M37207 Cry3Aa6 CrylIIA U10985 Cry3Ba1 CrylIIB X17123 Cry3Ba2 CrylIIB A07234 Cry3Bb1 CrylIIB2 M89794 Cry3Bb2 CrylIIC(b) U31633 Cry3Ca1 CrylIID X59797 Cry4Aa1 CrylVA Y00423 Cry4Aa2 CrylVA D00248 Cry4Ba1 CrylVB X07423 Cry4Ba2 CrylVB X07082 Cry4Ba3 CrylVB M20242 Cry4Ba4 CrylVB D00247 Cry5Aa1 CryVA(a) L07025 Cry5Ab1 CryVA(b) L07026 Cry5Ba1 PS86Q3 U19725 Cry6Aa1 **CryVIA** L07022 Cry6Ba1 **CryVIB** L07024 Cry7Aa1 CrylIIC M64478 Cry7Ab1 CrylIICb U04367 Cry8Aa1 CrylIIE U04364 Cry8Ba1 CrylIIG U04365 Cry8Ca1

CryIIIF U04366 Cry9Aa1 CryIG X58120 Cry9Aa2 CryIG X58534 Cry9Ba1 CryIX X75019 Cry9Ca1 CryIH Z37527 Cry9Da1 N141 D85560 Cry10Aa1 CryIVC M12662 Cry11Aa1 CryIVD M31737 Cry11Aa2 CryIVD M22860 Cry11Ba1 Jeg80 X86902 Cry12Aa1 CryVB L07027 Cry13Aa1 CryVC L07023 Cry14Aa1 CryVD U13955 Cry15Aa1 34kDa M76442 Cry16Aa1 cbm71 X94146 Cry17Aa1 cbm71 X99478 Cry18Aa1 CryBP1 X99049 Cry19Aa1 Jeg65 Y08920 Cry20Aa1 U82518 Cry21Aa1 I32932 Cry22Aa1 I34547 Cyt1Aa1 CytA X03182 Cyt1Aa2 CytA X04338 Cyt1Aa3 CytA Y00135 Cyt1Aa4 CytA M35968 Cyt1Ab1 CytM X98793 Cyt1Ba1 U37196 Cyt2Aa1 CytB Z14147 Cyt2Ba1 "CytB" U52043 Cyt2Ba2 "CytB" AF020789 Cyt2Ba3 "CytB" AF022884 Cyt2Ba4 "CytB" AF022885 Cyt2Ba5 "CytB" AF022886 Cyt2Bb1 U82519

_____ .sup.a Adapted from: http://epunix.biols.susx.ac.uk/Home/Neil.sub.-- Crickmore/Bt/index.html

Detailed Description Text - DETX (58):

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh et al., 1989; Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques involve annealing a primer which has crystal protein-specific sequences. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat denatured again. In either case the single stranded DNA is made fully double stranded by addition of second crystal protein-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into double stranded DNA, and transcribed once against with a polymerase such as T7 or SP6. The resulting products, whether **truncated or complete, indicate crystal protein**-specific sequences.

Detailed Description Text - DETX (227):

B. thuringiensis EG7231 was grown through sporulation in C2 medium with chloramphenicol (Cml) selection. The solids from this culture were recovered by centrifugation and washed with water. The toxin was purified by recrystallization from 4.0 M NaBr (Cody et al, 1992). The purified Cry3Bb was solubilized in 10 ml of 50mM KOH/100 mg Cry3Bb and buffered to pH 9.0 with 100 mM CAPS (pH 9.0). The soluble toxin was treated with trypsin at a weight ratio of 50 mg toxin to 1 mg trypsin. After 20 min of trypsin digestion the predominant protein visualized by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was 60 kDa. Further **digestion of the 60-kDa toxin** was not observed. FIG. 4 illustrates the Coomassie-stained Cry3Bb and Cry3Bb.60 following SDS-PAGE.

Detailed Description Text - DETX (242):

It has been reported in the literature that: treatment of Cry3A toxin protein with trypsin, an enzyme that cleaves proteins on the carboxyl side of available lysine and arginine residues, yields a stable cleavage product of 55 kDa from the 67 kDa native protein (Carroll et al., 1989). N-terminal sequencing of the 55 kDa product showed cleavage occurs at amino acid residue R158. The truncated Cry3A protein was found to retain the same level of insecticidal activity as the native protein. Cry3Bb toxin protein was also treated with trypsin. After digestion, the protein size decreased from 68 kDa, the molecular weight of the native Cry3Bb toxin, to 60 kDa. No further digestion was observed. N-terminal sequencing revealed the trypsin cleavage site of the **truncated toxin** (Cry3Bb.60) to be amino acid R159 in l.alpha.3,4 of Cry3Bb. Unexpectedly, the bioactivity of the **truncated Cry3Bb toxin** was found to increase.

Detailed Description Text - DETX (243):

Using this method, protease **digestion of a B. thuringiensis toxin** protein, a proteolytically sensitive site was identified on Cry3Bb, and a more highly active form of the protein (Cry3Bb.60) was identified. Modifications to this proteolytically-sensitive site by introducing an additional protease recognition site also resulted in the isolation of a biologically more active protein. It is also possible that removal of other protease-sensitive site(s) may improve activity. Proteolytically sensitive regions, once identified, may be modified or utilized to produce biologically more active toxins.

Detailed Description Text - DETX (245):

Treatment of solubilized Cry3Bb toxin protein with trypsin results in the isolation of a stable, **truncated Cry3Bb toxin** protein with a molecular weight of 60 kDa (Cry3Bb.60). N-terminal sequencing of Cry3Bb.60 shows the trypsin-sensitive site to be R159 in l.alpha.3,4 of the native toxin. Trypsin digestion results in the removal of helices 1-3 from the native Cry3Bb but also increases the activity of the toxin against SCRW larvae approximately four-fold.

Detailed Description Text - DETX (282):

The inventors have discovered that the first three helices of domain one could be cleaved from the rest of the **toxin by proteolytic digestion** of the loop between helices .alpha.3 and .alpha.4 (Cry3Bb.60). Initial efforts to truncate the cry3Bb gene to produce this shortened, though more active Cry3Bb molecule, failed. For unknown reasons, B. thuringiensis failed to synthesize this 60-kDa molecule. It was then reasoned that perhaps the first three helices of domain 1 did not have to be proteolytically removed, or equivalently, the protein did not have to be synthesized in this truncated form to take advantage of the Cry3Bb.60 design. It was observed that the protein Cry3A had a small amino acid near the l.alpha.3,4 that might impart greater flexibility in the loop region thereby permitting the first three helices of domain 1 to move out of the way, exposing the membrane-active region. By designing a Cry3Bb molecule with a glycine residue near this loop, the steric

hindrance of residues in the loop might be lessened. The redesigned protein, Cry3Bb.11032, has the amino acid change D165G, which replaces the larger aspartate residue (average mass of 115.09) with the smallest amino acid, glycine (average mass of 57.05). The activity of Cry3Bb.11032 is approximately 3-fold greater than that of the WT protein. In this way, the loop between helices .alpha.3 and .alpha.4 was rationally redesigned with a corresponding increase in the biological activity.

US-PAT-NO: **5973231**

DOCUMENT-IDENTIFIER: US 5973231 A
See image for Certificate of Correction

TITLE: Bacillus thuringiensis isolates, toxins, and genes for controlling certain coleopteran pests

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

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APPL-NO: 09/ 076193

DATE FILED: May 12, 1998

US-CL-CURRENT: 800/302, 435/252.3 , 435/419 , 435/468 , 536/23.71 , 800/279

ABSTRACT:

The subject invention concerns materials and methods useful in the control of pests and, particularly, plant pests. More specifically, the subject invention concerns novel genes and pesticidal toxins referred to as **86A1**(b) and 52A1(b). In preferred embodiments, the subject toxins are used for controlling flea beetles of the genus Phyllotreta. Using the genes described herein, the transformation of plants can be accomplished using techniques known to those skilled in the art. In addition, the subject invention provides toxin genes optimized for expression in plants.

15 Claims, 0 Drawing figures

Exemplary Claim Number: 12

---------- KWIC ---------

Abstract Text - ABTX (1):

The subject invention concerns materials and methods useful in the control of pests and, particularly, plant pests. More specifically, the subject invention concerns novel genes and pesticidal toxins referred to as **86A1**(b) and 52A1(b). In preferred embodiments, the subject toxins are used for controlling flea beetles of the genus Phyllotreta. Using the genes described herein, the

transformation of plants can be accomplished using techniques known to those skilled in the art. In addition, the subject invention provides toxin genes optimized for expression in plants.

US Patent No. - PN (1):

5973231

Brief Summary Text - BSTX (20):

B.t. isolate **PS86A1** is disclosed in the following, U.S. Pat. Nos. 4,849,217 (activity against alfalfa weevil); 5,208,017 (activity against corn rootworm); 5,286,485 (activity against lepidopterans); and 5,427,786 (activity against Phyllotreta genera). A gene from **PS86A1** was cloned into B.t. MR506, which is disclosed in U.S. Pat. No. 5,670,365 (activity against nematodes) and PCT international patent application publication no. WO93/04587 (activity against lepidopterans). The sequences of a gene and a **Cry6A (CryVIA)** toxin from **PS86A1** are disclosed in the following U.S. Pat. Nos. 5,186,934 (activity against Hypera genera); 5,273,746 (lice); 5,262,158 and 5,424,410 (activity against mites); as well as in PCT international patent application publication no. WO94/23036 (activity against wireworms). U.S. Pat. Nos. 5,262,159 and 5,468,636, disclose **PS86A1,** the sequence of a gene and toxin therefrom, and a generic formula for toxins having activity against aphids.

Brief Summary Text - BSTX (21):

B.t. isolate PS52A1 is disclosed by the following U.S. Pat. Nos. as being active against nematodes: 4,861,595; 4,948,734, 5,093,120, 5,262,399, 5,236,843, 5,322,932; and 5,670,365. PS52A1 is also disclosed in 4,849,217, supra, and PCT international patent application publication no. WO95/02694 (activity against Calliphoridae). The sequences of a gene and a nematode-active toxin from PS52A1 are disclosed in U.S. Pat. No. 5,439,881 and European patent application publication no. EP 0462721. PS52A1, the sequence of a gene and nematode-active toxin therefrom, and a generic formula for **CryVIA** toxins are disclosed in PCT international patent application publication no. WO 92/19739.

Brief Summary Text - BSTX (23):

Although B.t. strains **PS86A1** and PS52A1, and a gene and toxin therefrom, were known to have certain pesticidal activity, additional genes encoding active toxins from these isolates were not previously known in the art.

Brief Summary Text - BSTX (25):

The subject invention provides novel genes encoding pesticidal toxins. Preferred, novel toxin genes of the subject invention are designated **86A1**(b)

and 52A1(b). These genes encode toxins that are active against plant pests, preferably insects, preferably coleopterans, and most preferably flea beetles of the genus Phyllotreta.

Brief Summary Text - BSTX (30):

SEQ ID NO. 1 is a forward oligonucleotide probe for 52A1(b) and **86A1**(b).

Brief Summary Text - BSTX (31):

SEQ ID NO. 2 is a nucleotide sequence of a gene encoding the **86A1**(b) toxin.

Brief Summary Text - BSTX (32):

SEQ ID NO. 3 is an amino acid sequence of the **86A1**(b) toxin.

Brief Summary Text - BSTX (38):

The subject invention provides novel genes encoding pesticidal toxins. Preferred, novel toxin genes of the subject invention are designated **86A1**(b) and 52A1(b). These genes encode toxins that are active against (which can be used to control, or which are toxic to, or which are lethal to) plant pests, preferably insects, preferably coleopterans, and most preferably flea beetles of the genus Phyllotreta. The use of the subject genes and toxins for controlling other pests, such as pests of the genus Psylliodes, is also contemplated.

Brief Summary Text - BSTX (41):

Characteristics of Bacillus thuringiensis isolates **PS86A1** and PS52A1, such as colony morphology, inclusion type, and the sizes of alkali-soluble proteins (by SDS-PAGE), have been disclosed in, for example, U.S. Pat. No. 5,427,786 and published PCT application WO 95/02694, respectively.

Brief Summary Text - BSTX (46):

Fragments of the genes and toxins specifically exemplified herein which retain the pesticidal activity of the exemplified toxins are within the scope of the subject invention. Genes and toxins useful according to the subject invention include not only the full length sequences but also fragments of these sequences which retain the characteristic pesticidal activity of the toxins specifically exemplified herein.

Brief Summary Text - BSTX (48):

Genes can be modified, and variations of genes may be readily constructed,

as would be known to one skilled in the art. Standard techniques are available for making point mutations. The use of site-directed mutagenesis is known in the art. Fragments of the subject genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or can be used to systematically cut off nucleotides from the ends of these genes. Useful genes may be obtained using a variety of restriction enzymes. Proteases may be used to directly obtain active **fragments of these toxins**.

Brief Summary Text - BSTX (51):

There are a number of methods for obtaining the pesticidal toxins of the instant invention. For example, antibodies to the pesticidal toxins disclosed and claimed herein can be used to identify and isolate toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the toxins which are most constant and most distinct from other Bacillus toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic activity by immunoprecipitation, enzyme linked immunosorbent assay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent **toxins or fragments of these toxins,** can readily be prepared using standard procedures in this art.

Brief Summary Text - BSTX (55):

Full length B.t. toxins can be expressed and then converted to active, truncated forms through the addition of appropriate reagents and/or by growing the cultures under conditions which result in the truncation of the proteins through the fortuitous action of endogenous proteases. In an alternative embodiment, the full length toxin may undergo other modifications to yield the active form of the toxin. Adjustment of the solubilization of the toxin, as well as other reaction conditions, such as pH, ionic strength, or redox potential, can be used to effect the desired modification of the toxin. **Truncated toxins** of the subject invention can be obtained by treating the crystalline .delta.-endotoxin of **Bacillus thuringiensis** with a serine protease such as bovine trypsin at an alkaline pH and preferably in the absence of .beta.-mercaptoethanol.

Brief Summary Paragraph Table - BSTL (1):

TABLE 1 _____ Repository Culture Accession No. Deposit date _____ B.t. var. wuhanensis **PS86A1** NRRL B-18400 August 16, 1988 B.t. var. wuhanensis PS52A1 NRRL B-18245 July 28, 1987 _____

Detailed Description Text - DETX (8):

Molecular Cloning, Expression, and Sequencing of Novel Toxin Genes from Bacillus thuringiensis Strains PS52A1 and **PS86A1**

Detailed Description Text - DETX (9):

Total cellular DNA was prepared from PS52A1 and **PS86A1** Bacillus thziringiensis (B.t.) cells grown at 30.degree. C. to an optical density of 1.0 at 600 nm. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/mL lysozyme in 0.3M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation at 37.degree. C. for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1M NaCl, 0.1% SDS, 0.1M Tris-Cl [pH 8.0] were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform(1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by centrifugation. The pellet was resuspended in TE buffer (10 mM Tris-Cl [pH 8.0], 1 mM EDTA) and RNase was added to a final concentration of 50 .mu.g/mL. After incubation at 37.degree. C. for 1 hour, the solution was extracted once each with phenol:chloroform(1:1) and TE-saturated chloroform. From the aqueous phase, DNA was precipitated by the addition of one-tenth volume 3M NaOAc and two volumes ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer.

Detailed Description Text - DETX (10):

Plasmid DNA was also prepared from B.t. strain **PS86A1**. The B.t. cells were grown at 30.degree. C. to an optical density of 1.0 at 600 nm. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/mL lysozyme in 0.3M sucrose, 25 mM Tris-Cl [pH 8.0], 25 mM EDTA). After incubation on ice for 30 minutes, ten volumes of lysis buffer (0.085M NaOH, 0.1% SDS in TE buffer) were added. The lysate was rocked gently at room temperature for 30 minutes. One-half volume 3M KOAc was added to the suspension for incubation at 4.degree. C. overnight. Nucleic acids were precipitated with one volume isopropanol and pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer. The DNA suspension was further purified by extraction once with phenol:chloroform (1:1). DNA in the aqueous phase was precipitated by the addition of one-tenth volume 3M NaOAc and one volume of isopropanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE buffer. CsCl was added at equal weight to volume of DNA solution, and ethidium bromide was added to a final concentration of 0.5 mg/mL. The plasmid DNA was separated from the extraneous nucleic acids by overnight ultracentrifugation. The recovered plasmid band was extracted five times with excess water-saturated butanol, and dialyzed against TE buffer. DNA was precipitated, pelleted, washed, dried and resuspended in TE buffer as described previously. Based on N-terminal amino acid sequencing data of the **PS86A1** 45 kDa polypeptide, the following "forward" oligonucleotide of sequence (SEQ ID NO. 1) was synthesized for use in Southern hybridizations:

Detailed Description Text - DETX (12):

PS86A1 total cellular and plasmid DNA were digested with selected restriction endonucleases, electrophoresed on an agarose gel, subsequently blotted onto a nylon membrane, and immobilized by "baking" the membrane at 80.degree. C. Restriction fragment length polymorphism (RFLP) analysis was

performed using the oligonucleotide probe described above. Southern blots were hybridized overnight in 6.times. SSPE, 5.times. Denhardt's solution, 0.1 mg/mL single stranded carrier DNA and 0.1% SDS at 37.degree. C. The blots were then washed in 1.times. SSPE, 0.1% SDS at 37.degree. C., air-dried, then exposed to X-ray film. Autoradiography identified an approximately 6.6 kbp Xba I band in both the total cellular and plasmid DNA blots that was theorized to contain all or part of the PS86B1(b) toxin gene.

Detailed Description Text - DETX (13):

The approximately 6.6 kbp Xba I fragment was cloned into pHTBluell (an E. coli/B. thuringiensis shuttle vector composed of pBluescript II SK- (Stratacene, La Jolla, Calif.) and the replication origin from a resident B.t. plasmid Lereclus et al. [1989] FEMS Microbiology Letters 60:211-218]). Polymerase chain reaction (PCR) mapping to determine if the fragment contained the full-length gene was conducted using the "forward" oligonucleotide primer described previously and vector primers. The "forward" primer combined with vector primer T7 resulted in amplification of only an approximately 400 bp-sized fragment, instead of the approximately 1.0 kbp gene expected to encode a protein of 45 kDa length. This established that only approximately one-third of the **PS86A1**(b) toxin gene was cloned. Further verification was provided by dideoxynucleotide sequencing (Sanger et al. [1977] Proc. Natl. Acad. Sci. USA 74:5463-5467) using Sequenase (US Biochemical, Cleveland, Ohio) on the subgene construct. The PCR fragment was subsequently radiolabelled with .sup.32 P and used as a probe in standard hybridizations of Southern blots and gene libraries of **PS86A1** and PS52A1 total cellular DNA.

Detailed Description Text - DETX (14):

A gene library was constructed from **PS86A1** total cellular DNA partially digested with Sau3A I. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column (Schleicher and Schuell, Keene, N.H.), and recovered by ethanol precipitation. The Sau3A I inserts were ligated into BamHI-digested LambdaGem-11 (Promega, Madison, Wis.). Recombinant phage were packaged and plated on E. coli KW251 (Promega, Madison, Wis.) cells. Plaques were screened by transfer of recombinant phage DNA to filters and hybridization with the PCR probe described previously. Hybridizationwas carried out overnight at 37.degree. C. in a solution consisting of 6.times. SSPE, 5.times. Denhardt's solution, 0.1 mg/mL single stranded carrier DNA, and 0.1% SDS. The filters were subsequently washed in 1.times. SSPE and 0.1% SDS at 37.degree. C., air-dried, and then exposed to X-ray film. Hlybridizing phage were plaque-purified and used to infect liquid cultures of E. coli KW251 cells for isolation of DNA by standard procedures (Maniatis et al. [1982] Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Southern blotting of plaque-purified hybridizing phage DNA digested with selected restriction endonucleases using the PCR-amplified probe and washing conditions as described above revealed an approximately 2.3 kbp EcoR V+Sal I fragment believed to contain the **PS86A1**(b) gene.

Detailed Description Text - DETX (15):

For subcloning the **PS86A1**(b) gene encoding the approximately 45 kDa toxin, preparative amounts of phage DNA were divested with EcoRV and SalI. The approximately 2.3 kbp band was ligated into SmaI+SalI-digested pHTBlueII. The ligation mix was used to transform frozen, competent E. coli NM522 cells (ATCC 47000). .beta.-galactosidase-negative transformants were screened by restriction digestion of alkaline lysate plasmid miniprep DNA. The desired plasmid construct, pMYC2344, contains the **PS86A1**(b) toxin gene. pMYC2344 was introduced into the acrystalliferous (Cry-) B.t. host, CryB (A. Aronson, Purdue University, West Lafayette, Ind.) by electroporation. Expression of the toxin was demonstrated by visualization of crystal formation under microscopic examination, and SDS-PAGE analysis. Gene construct pMYC2344 in B.t. is designated MR509.

Detailed Description Text - DETX (16):

A sequence of the **86A1**(b) gene is shown in SEQ ID NO. 2. A deduced amino acid sequence for the **86A1**(b) toxin is shown in SEQ ID NO. 3.

Detailed Description Text - DETX (17):

The **PS86A1**(b) probes, hybridization, and washing conditions were also used to clone a related gene, PS52A1(b), from Bacillus thuringiensis strain PS52A1. A gene library was constructed by partially digesting PS52A1 total cellular DNA with Sau3A I. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 9.3 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, purified on an Elutip-D ion exchange column, and recovered by ethanol precipitation. The Sau3A I inserts were ligated into BamHI-digested LambdaGem-11. Recombinant phage were packaged and plated on E. coli KW251 cells. Plaques were screened by hybridization with the PCR probe described previously. Hybridizing phage were plaque-purified and used to infect liquid cultures of E. coli KW251 cells for isolation of DNA by standard procedures. Southern blotting of plaque-purified hybridizing phage DNA digested with selected restriction endonucleases using the PCR probe revealed an approximately 2.3 kbp EcoRV+SalI fragment believed to contain the PS52A1(b) gene.

Detailed Description Text - DETX (21):

Bioassay of the MR509/**86A1**(b) Toxin Against Phyllotreta

Detailed Description Text - DETX (23):

Several treatments showed reduced plant damage relative to untreated and CryB (a crystal-minus B.t. strain) controls. It was determined that the approximately 45 kda protein from MR509 was highly active against the tested Phyllotreta cruciferae pests; this toxin is referred to as the **86A1**(b) gene.

Detailed Description Text - DETX (25):

Further Bioassays--MR509/**86A1**(b) and MR510/52A1(b) Against Phyllotreta spp.

Detailed Description Text - DETX (37):

In a preferred embodiment of the subject invention, plants will be transformed with genes wherein the codon usage has been optimized for plants. See, for example, U.S. Pat. No. No. 5,380,831. Also, advantageously, DNA encoding a **truncated toxin** will be used. The **truncated toxin** typically will encode about 55% to about 80% of the full length toxin. Methods for creating synthetic Bacillus genes for use in plants are known in the art.

US-PAT-NO: **5866421**

DOCUMENT-IDENTIFIER: US 5866421 A

TITLE: Enhanced expression in a plant plastid

DATE-ISSUED: February 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
McBride; Kevin E.	Davis	CA	N/A	N/A
Stalker; David M.	Davis	CA	N/A	N/A

APPL-NO: 08/ 593205

DATE FILED: January 29, 1996

PARENT-CASE:

INTRODUCTION

This application is a continuation of application PCT/US95/02901 filed Mar. 10, 1995 and a continuation-in-part of U.S. application Ser. No. 08/209,649 filed Mar. 11, 1994 issued as U.S. Pat. No. 5,545,817.

US-CL-CURRENT: 435/419, 435/320.1 , 435/440 , 435/468

ABSTRACT:

Novel compositions and methods useful for genetic engineering of plant cells to provide increased expression in the plastids of a plant or plant cell of a protein which produces a phenotype which is present when the plant or plant cell is grown in the absence of means for selecting transformed cells. Expression of the Bacillus thuringiensis bacterial protoxin in a plant chloroplast is exemplified.

9 Claims, 1 Drawing figures

Exemplary Claim Number: 1,6

Number of Drawing Sheets: 1

---------- KWIC ---------

US Patent No. - PN (1):

5866421

Brief Summary Text - BSTX (12):

In particular, there is a continuing need to introduce newly discovered or alternative **Bacillus thuringiensis** genes into crop plants. Cry proteins (d-endotoxins) from **Bacillus thuringiensis** have potent insecticidal activity against a number of Lepidopteran, Dipteran, and Coleopteran insects. These proteins are classified CryI to CryV, based on amino acid sequence homology and insecticidal activity. Most CryI proteins are synthesized as protoxins (ca. 130-140 kDa) then solubilized and proteolytically processed into active **toxin fragments** (ca. 60-70 kDa).

Brief Summary Text - BSTX (13):

The poor expression of the protoxin genes from the nucleus of plants has heretofore required the use of `truncated` versions of these genes. The truncated versions code only for the active **toxin fragments**. Other attempts to increase the expression efficiency have included resynthesizing the **Bacillus thuringiensis** toxin genes to utilize plant preferred codons. Many problems can arise in such extensive reconstruction of these large cry genes (approximately 3.5 Kb), and the process is both laborious and expensive.

Brief Summary Text - BSTX (22):

By this invention the insecticidal **Bacillus thuringiensis** toxin is produced in plastids of a plant cell from the native DNA encoding sequence, with enhanced levels of expression of an insect resistant phenotype, as measured by insect feeding assays. The native **Bacillus thuringiensis** DNA encoding sequence may be the truncated version specific to the active fragment. This invention also provides the expression of the **Bacillus thuringiensis toxin from the non-truncated** sequence which encodes the protoxin.

Detailed Description Text - DETX (23):

A synthetic **Bacillus thuringiensis** gene is placed in the same expression construct as the protoxin gene. The synthetic gene is designed to have tobacco RuBPCO small subunit codon usage, with an overall increase in the guanine plus cytosine content to 55% (with respect to the native gene content of 39%), and has been truncated to leave only those sequences which enode the active **fragment of the toxin**. Such a gene is known to provide optimal expression from the plant nuclear genome. Both the bacterial gene which has been resynthesized for increased expression from plant nuclear transformation and the non-resynthesized, non-truncated wild-type gene to the protoxin are introduced via a chloroplast transformation vector (FIG. 1).

US-PAT-NO: **5753492**

DOCUMENT-IDENTIFIER: US 5753492 A
See image for Certificate of Correction

TITLE: Genes encoding nematode-active toxins from Bacillus thuringiensis strains

DATE-ISSUED: May 19, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schnepf; H. Ernest	San Diego	CA	N/A	N/A
Schwab; George E.	La Jolla	CA	N/A	N/A
Payne; Jewel	Davis	CA	N/A	N/A
Narva; Kenneth E.	San Diego	CA	N/A	N/A
Foncerrada; Luis	Vista	CA	N/A	N/A

APPL-NO: 08/ 316301

DATE FILED: September 30, 1994

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This is a division of application Ser. No. 07/871,510, filed on Apr. 23, 1992, now abandoned; which is a continuation-in-part of application Ser. No. 07/693,018, filed on May 3, 1991, now abandoned; which is a continuation-in-part of Ser. No. 07/565,544, filed on Aug. 10, 1990, now abandoned; which is a continuation-in-part of application Ser. No. 07/084,653, filed on Aug. 12, 1987, now U.S. Pat. No. 4,948,734. This is also a continuation-in-part of application Ser. No. 07/830,050, filed on Jan. 31, 1992, now abandoned.

US-CL-CURRENT: 435/252.3, 435/325 , 435/419 , 536/23.71

ABSTRACT:

This invention concerns genes or gene fragments which have been cloned from novel Bacillus thuringiensis isolates which have nematicidal activity. These genes or gene fragments can be used to transform suitable hosts for controlling nematodes.

8 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

US Patent No. - PN (1):

5753492

Brief Summary Text - BSTX (11):

One aspect of the of the subject invention is the discovery of two groups of B.t.-derived nematode-active toxins. One group (CryV) is exemplified by the gene expression products of PS17, PS33F2 and PS63B, while the other group (**CryVI**) is exemplified by the gene expression products of PS52A1 and PS69D1. The organization of the toxins within each of the two groups can be accomplished by sequence-specific motifs, overall sequence similarity, immunoreactivity, and ability to hybridize with specific probes.

Brief Summary Text - BSTX (64):

One aspect of the subject invention concerns the discovery of generic chemical formulae which describe toxins having activity against nematodes. Two formulae are provided: one which pertains to nematicidal toxins having molecular weights of between about 45 kDa and 65 kDa, and the other pertains to larger nematicidal proteins having molecular weights from about 65 kDa to about 155 kDa. These formulae represent two different categories of B.t. .delta.-endotoxins, each of which has activity against nematodes. The formula describing smaller proteins describes many **CryVI** proteins, while the formula describing larger proteins describes many CryV proteins. A description of these two formulae is as follows:

Brief Summary Text - BSTX (106):

Further guidance for characterizing the nematicidal toxins of the subject invention is provided in Tables 3 and 4, which demonstrate the relatedness among toxins within each of the above-noted groups of nematicidal toxins (CryV and **CryVI**). These tables show a numeric score for the best matching alignment between two proteins that reflects: (1) positive scores for exact matches, (2) positive or negative scores reflecting the likelihood (or not) of one amino acid substituting for another in a related protein, and (3) negative scores for the introduction of gaps. A protein sequence aligned to itself will have the highest possible score--i.e., all exact matches and no gaps. However, an unrelated protein or a randomly generated sequence will typically have a low positive score. Related sequences have scores between the random background score and the perfect match score.

Brief Summary Text - BSTX (108):

Tables 3 and 4 show the pairwise alignments between the indicated amino acids of the two classes of nematode-active proteins CryV and **CryVI** and representatives of dipteran (CryIV; Sen, K. et al. [1988] Agric. Biol. Chem.

52:873-878), lepidopteran and dipteran (CryIIA; Widner and Whiteley [1989] J. Bacteriol. 171:965-974), lepidopteran (CryIA(c); Adang et al. [1981] Gene 36:289-300), and coleopteran (CryhIIIA; Herrnstadt et al. [1987] Gene 57:37-46) proteins.

Brief Summary Text - BSTX (119):

It should be apparent to a person skilled in this art that genes coding for nematode-active toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described below. These genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active **fragments of these toxins**.

Brief Summary Text - BSTX (120):

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from B.t. isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the nematode-active toxins of the instant invention which occur in nature. For example, antibodies to the nematode-active toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the portions of the nematode-active toxins which are most constant and most distinct from other B.t. toxins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic nematicidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent **toxins, or fragments of these toxins,** can readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

Brief Summary Text - BSTX (135):

The **toxin genes or gene fragments** exemplified according to the subject invention can be obtained from nematode-active B. thuringiensis (B.t.) isolates designated PS17, PS33F2, PS63B, PS52A1, and PS69D1. Subcultures of the E. coli host harboring the toxin genes of the invention were deposited in the permanent collection of the Northern Research Laboratory, U.S. Department of Agriculture, Peoria, Ill., USA. The accession numbers are as follows:

Brief Summary Text - BSTX (138):

The novel B.t. genes or gene **fragments of the invention encode t xins** which

show activity against tested nematodes. The group of diseases described generally as helminthiasis is due to infection of an animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes wide-spread and often times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagostomum, Chabertia, Trichuris, Strongylus, Tfhchonema, Dictyocaulus, Capillaria, Heterakis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris, Caenorhabditis and Parascaris. Certain of these, such as Nematodirus, Cooperia, and Oesophagostomum, attack primarily the intestinal tract, while others, such as Dictyocaulus are found in the lungs. Still other parasites may be located in other tissues and organs of the body.

Brief Summary Text - BSTX (146):

The **toxin genes or gene fragments** of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the nematicide. With suitable hosts, e.g., Pseudomonas, the microbes can be applied to the situs of nematodes where they will proliferate and be ingested by the nematodes. The result is a control of the nematodes. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the B.t. toxin.

Brief Summary Text - BSTX (147):

Where the B.t. **toxin gene or gene fragment** is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the nematicide from environmental degradation and inactivation.

Brief Summary Text - BSTX (149):

A wide variety of ways are known and available for introducing the B.t. genes or gene **fragments expressing the toxin** into the microorganism host under conditions which allow for stable maintenance and expression of the gene. The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired

organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for nematicidal activity.

Brief Summary Text - BSTX (154):

Treatment of the microbial cell, e.g., a microbe containing the B.t. **toxin gene or gene fragment,** can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W. H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

Detailed Description Text - DETX (15):

In addition, internal amino acid sequence data were derived for PS63B. The **toxin protein was partially digested** with Staphylococcus aureus V8 protease (Sigma Chem. Co., St. Louis, Mo.) essentially as described (Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and U. K. Laemmli [1977] J. Biol. Chem. 252:1102). The digested material was blotted onto PVDF membrane and a ca. 28 kDa limit peptide was selected for N-terminal sequencing as described above. The sequence obtained was:

Detailed Description Text - DETX (54):

These primers were used in standard polymerase chain reactions (Cetus Corporation) to amplify an approximately 460 bp **fragment of the 63B toxin** gene for use as a DNA cloning probe. Standard Southern blots of total cellular DNA from PS63B were hybridized with the radiolabeled PCR probe. Hybridizing bands included an approximately 4.4 kbp XbaI fragment, an approximately 2.0 kbp HindIII fragment, and an approximately 6.4 kbp SpeI fragment.

US-PAT-NO: **5489432**

DOCUMENT-IDENTIFIER: US 5489432 A
See image for Certificate of Correction

TITLE: Bacillus thuringiensis isolates active against cockroaches and genes encoding cockroach-active toxins

DATE-ISSUED: February 6, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Payne; Jewel M.	San Diego	CA	N/A	N/A
Kennedy; M. Keith	Racine	WI	N/A	N/A
Randall; John B.	Racine	WI	N/A	N/A
Brower; David O.	Racine	WI	N/A	N/A
Schnepf; H. Ernest	San Diego	CA	N/A	N/A

APPL-NO: 08/ 129609

DATE FILED: September 30, 1993

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This is a continuation-in-part of application Ser. No. 07/958,551, filed Oct. 19, 1992, now U.S. Pat. No. 5,302,387 which is a continuation-in-part of application Ser. No. 07/788,654, filed Nov. 6, 1991, now abandoned.

US-CL-CURRENT: 424/405, 424/93.461

ABSTRACT:

The subject invention concerns a novel microbe and genes encoding novel toxin proteins with activity against cockroaches. Cockroaches are common house pests, and they create problems in hospitals, the food industry and in agriculture. The novel **Bacillus thuringiensis** microbe of the invention is referred to as B.t. PS185L8. The subject invention also concerns the use of B.t. PS201T6 to control cockroaches. A **truncated form of a toxin** obtained from PS201T6 having particular activity to cockroaches is also claimed for use in controlling the pest. The spores or crystals of the two microbes, or mutants thereof, are useful to control cockroaches in various environments. The genes of the invention can be used to transform various hosts wherein the novel toxic proteins can be expressed.

2 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Abstract Text - ABTX (1):

The subject invention concerns a novel microbe and genes encoding novel toxin proteins with activity against cockroaches. Cockroaches are common house pests, and they create problems in hospitals, the food industry and in agriculture. The novel **Bacillus thuringiensis** microbe of the invention is referred to as B.t. PS185L8. The subject invention also concerns the use of B.t. PS201T6 to control cockroaches. A **truncated form of a toxin** obtained from PS201T6 having particular activity to cockroaches is also claimed for use in controlling the pest. The spores or crystals of the two microbes, or mutants thereof, are useful to control cockroaches in various environments. The genes of the invention can be used to transform various hosts wherein the novel toxic proteins can be expressed.

US Patent No. - PN (1):

5489432

US-PAT-NO: **5468636**

DOCUMENT-IDENTIFIER: US 5468636 A
See image for Certificate of Correction

TITLE: Bacillus thuringiensis for controlling pests in the family aphididae

DATE-ISSUED: November 21, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Payne; Jewel M.	San Diego	CA	N/A	N/A
Cannon; Raymond J. C.	Sittingbourne	N/A	N/A	GB
Schnepf; H. Ernest	San Diego	CA	N/A	N/A
Schwab; George E.	La Jolla	CA	N/A	N/A

APPL-NO: 08/ 147189

DATE FILED: November 3, 1993

PARENT-CASE:

This is a division of application Ser. No. 07/935,310, filed Aug. 24, 1992, now U.S. Pat. No. 5,262,159

US-CL-CURRENT: 435/252.3, 424/93.461 , 435/252.31 , 435/252.33 , 536/23.71

ABSTRACT:

The subject invention concerns Bacillus thuringiensis isolates designated B.t. PS157C1, B.t. **PS86A1,** and B.t. PS75J1, which are active against aphid pests. Thus, these isolates, or variants thereof, can be used to control such pests. Further, genes encoding novel .delta.-endotoxins can be removed from these isolates and transferred to other host microbes, or plants. Expression of the .delta.-endotoxins in microbe hosts results in the control of aphid pests, whereas transformed plants become resistant to aphid pests.

5 Claims, 2 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

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Abstract Text - ABTX (1):

The subject invention concerns Bacillus thuringiensis isolates designated B.t. PS157C1, B.t. **PS86A1,** and B.t. PS75J1, which are active against aphid pests. Thus, these isolates, or variants thereof, can be used to control such pests. Further, genes encoding novel .delta.-endotoxins can be removed from these isolates and transferred to other host microbes, or plants. Expression of the .delta.-endotoxins in microbe hosts results in the control of aphid pests, whereas transformed plants become resistant to aphid pests.

US Patent No. - PN (1):

5468636

Brief Summary Text - BSTX (7):

The subject invention concerns Bacillus thuringiensis isolates which have aphidicidal properties. More specifically, the subject invention concerns the use of Bacillus thuringiensis isolates designated B.t. PS157C1 (also known as B.t. MT104), B.t. **PS86A1,** and B.t. PS75J1 to control aphids in the environment.

Brief Summary Text - BSTX (9):

Specifically exemplified herein is the cloning of gene **86A1** obtainable from B.t. **PS86A1**. Using the teachings of the subject invention, a person skilled in the art could identify other B.t. aphidicidal toxins, as well as the genes which code for such toxins.

Brief Summary Text - BSTX (14):

SEQ ID NO. 1 is the DNA sequence of a gene of B.t. **PS86A1**.

Brief Summary Text - BSTX (15):

SEQ ID NO. 2 is the amino acid sequence of the toxin encoded by a gene of B.t. **PS86A1**.

Brief Summary Text - BSTX (21):

SEQ ID NO. 8 is an N-terminal amino acid sequence of **86A1**.

Brief Summary Text - BSTX (22):

SEQ ID NO. 9 is an oligonucleotide probe designed from SEQ ID NO. 3, designated **86A1**-A.

Detailed Description Text - DETX (3):

Specifically exemplified herein are the isolates designated B.t. PS157C1, B.t. **PS86A1,** and B.t. PS75J1. Also specifically exemplified is the toxin designated **86A1** and the gene which codes for this toxin. The discovery described in the subject application also enables a person skilled in the art to identify other toxins (and genes coding for these toxins) having aphidicidal activity. The toxins of the subject invention are characterized as being aphidicidal and having one or more of the following characteristics:

Detailed Description Text - DETX (4):

1. A high degree of amino acid homology with toxin **86A1**.

Detailed Description Text - DETX (8):

5. Immunoreactivity to an antibody raised to toxin **86A1**.

Detailed Description Text - DETX (20):

Aphidicidal toxins of the subject invention are specifically exemplified herein by the toxin designated **86A1**. The subject invention further comprises equivalent toxins (and nucleotide sequences coding for equivalent toxins) having the same or similar biological activity of **86A1**. These equivalent toxins may have amino acid homology with the toxin disclosed and claimed herein. This amino acid homology will typically be greater than 50%, preferably be greater than 75%, and most preferably be greater than 90%. The amino acid homology will be highest in certain critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 2 provides a listing of examples of amino acids belonging to each class.

Detailed Description Text - DETX (25):

This formula is exemplified in the current application by the specific toxin **86A1**.

Detailed Description Text - DETX (26):

It should be apparent to a person skilled in this art that genes coding for aphidicidal toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described herein. Alternatively, these genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active **fragments of these toxins**.

Detailed Description Text - DETX (27):

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from B.t. isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the aphidicidal toxins of the instant invention which occur in nature. For example, antibodies to the aphidicidal toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. These antibodies can then be used to specifically identify equivalent toxins with the characteristic aphidicidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent **toxins, or fragments of these toxins,** can readily be prepared using standard procedures in this art.

Detailed Description Text - DETX (59):

From this sequence, the following oligonucleotide probe was designed: ##STR1## This probe was designated as **86A1**-A.

Detailed Description Text - DETX (61):

Molecular Cloning of Gene Encoding a Novel Toxin from Bacillus thuringiensis Strain **PS86A1**

Detailed Description Text - DETX (62):

Total cellular DNA was prepared from **PS86A1** cells grown to an optical density, at 600 nm, of 1.0. Cells were pelleted by centrifugation and resuspended in protoplast buffer (20 mg/ml lysozyme in 0.3M sucrose, 25 mM Tris-Cl, pH 8.0, 25 mM EDTA). After incubation at 37.degree. C. for 1 hour, protoplasts were lysed by two cycles of freezing and thawing. Nine volumes of a solution of 0.1M NaCl, 0.1% SDS, 0.1M Tris-Cl were added to complete lysis. The cleared lysate was extracted twice with phenol:chloroform (1:1). Nucleic acids were precipitated with two volumes of ethanol and pelleted by

centrifugation. The pellet was resuspended in 10 mM Tris-Cl, 1 mM EDTA (TE), pH 8.0, and RNAse was added to a final concentration of 50 .mu.g/ml. After incubation at 37.degree. C. for 1 hour, the solution was extracted once each with phenol:chloroform (1:1) and TE-saturated chloroform. DNA was precipitated from the aqueous phase by the addition of one-tenth volume of 3M NaOAc and two volumes of ethanol. DNA was pelleted by centrifugation, washed with 70% ethanol, dried, and resuspended in TE.

Detailed Description Text - DETX (63):

Restriction fragment length polymorphism (RFLP) analyses were performed by standard hybridization of southern blots of **PS86A1** DNA with the .sup.32 P-labeled oligonucleotide probe designated as **86A1**-A.

Detailed Description Text - DETX (65):

A gene library was constructed from **PS86A1** DNA partially digested with Sau3A. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 6.6 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column. The Sau3A inserts were ligated into BamHI-digested LambdaGem-11 (Promega, Madison, Wis.). Recombinant phage were packaged and plated on E. coli KW251 cells (Promega). Plaques were screened by hybridization with the radiolabeled **86A1**-A oligonucleotide probe. Hybridizing phage were plaque-purified and used to infect liquid cultures of E. coli KW251 cells for isolation of phage DNA by standard procedures (Maniatis et al. [1982] Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). For subcloning, preparative amounts of DNA were digested with EcoRI and SalI, and electrophoresed on an agarose gel. The approximately 2.9 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. The purified DNA insert was ligated into EcoRI+SalI-digested pHTBluell (an E. coli/B.t. shuttle vector comprised of pBlueScript S/K (Stratagene, San Diego, Calif.) and the replication origin from a resident B.t. plasmid (D. Lereclus et al. [1989] FEMS Microbiol. Lett. 60:211-218). The ligation mix was used to transform frozen, competent E. coli NM522 cells (ATCC 47000). Transformants were plated on LB agar (Maniatis et al., supra) containing ampicillin, isopropyl-(.beta.)-D-thiogalactoside (IPTG), and 5-bromo-4-chloro-3-indolyl-(.beta.)-D-galactoside (XGAL). Plasmids were purified from putative recombinants by alkaline lysis (Maniatis et al., supra) and analyzed by electrophoresis of EcoRI and SalI digests on agarose gels. The desired plasmid construct, pMYC2320, contains the toxin gene of the invention. See FIG. 2. The DNA sequence of this gene is shown in SEQ ID NO. 1. The toxin expressed by this gene is shown in SEQ ID NO. 2.

Detailed Description Paragraph Table - DETL (1):

TABLE 1

Comparison of B. t. PS75J1, B. t. **PS86A1,** B. t. PS157C1, B. t. s. d., and . t.

HD-1 B. t. PS75J1 B. t. **PS86A1** B. t. PS157C1 B. t. HD-1 B. t. s. d.

Inclusions: Amorphic Multiple Flat square and Bipyramid Flat square and bipyramid Approximate 81,000 58,000 130,000 130,000 72,000 molecular wt. of 79,000 45,000 72,000 68,000 64,000 proteins by 75,000 64,000 SDS-PAGE 63,000 Serotype wuhenensis wuhenensis morrisoni kurstaki morrisoni Host range Aphid, Mite, Aphid, Mite, Aphid, Lepidoptera Coleoptera Coleoptera Coleoptera Lepidoptera, (CPB) (AW, CRW, (AW, CRW, Coleoptera RFB) RFB) (CPB)

CPB
= Colorado Potato Beetle; AW = Alfalfa Weevil; CRW = Corn Rootworm; RFB = Rape Flea Beetle

Detailed Description Paragraph Table - DETL (2):

Culture Accession No. Deposit Date
B. t. PS75J1 NRRL B-18781 March 7, 1991
B. t. **PS86A1** NRRL B-18400 August 16, 1988 B. t. PS157C1 (a.k.a. MT104) NRRL B-18240 July 17, 1987 E. coli NM522 [pMYC2320] NRRL B-18769 February 14, 1991

Detailed Description Paragraph Table - DETL (6):

TABLE 3 Isolate Percent Mortality
B. t. PS157C1 100 B. t. **PS86A1** 90 B. t. PS75J1 100 Control 0

Detailed Description Paragraph Table - DETL (7):

SEQUENCE LISTING (1) GENERAL INFORMATION: (iii) NUMBER OF SEQUENCES: 15 (2) INFORMATION FOR SEQ ID NO:1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1425 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (genomic) (iii) HYPOTHETICAL: NO (iv) ANTI-SENSE: NO (vi) ORIGINAL SOURCE: (A) ORGANISM: BACILLUS THURINGIENSIS (C) INDIVIDUAL ISOLATE: **PS86A1** (vii) IMMEDIATE SOURCE: (A) LIBRARY: E. coli NM522[pMYC2320] (ix) FEATURE: (A) NAME/KEY: mat-peptide (B) LOCATION: 1..1425 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGATTATTGATAGTAAAACGACTTTACC
TAGACATTCACTTATTCATACAATTAAATTA60
AATTCTAATAAGAAATATGGTCCTGGTGATATGACTAATGGAAATCAATTTATTATTTCA120
AAACAAGAATGGGCTACGATTGGAGCATATATTCAGACTGGATTAGGTTTACCAGTAAAT18
GAA
CAACAATTAAGAACACATGTTAATTTAAGTCAGGATATATCAATACCTAGTGATTTT240
TCTCAATTATATGATGTTTATTGTTCTGATAAAACTTCAGCAGAATGGTGGAATAAAAAT300
TTATATCCTTTAATTATTAAATCTGCTAATGATATTGCTTCATATGG TTTTAAAGTTGCT360
GGTGATCCTTCTATTAAGAAAGATGGATATTTTAAAAAATTGCAAGATGAATTAGATAAT420
ATTGTTGATAATAATTCCGATGATGATGCAATAGCTAAAGCTATTAAAGATTTTAAAGCG480
CGATGTGGTATTTTAATTAAA GAAGCTAAACAATATGAAGAAGCTGCAAAAAATATTGTA540

ACATCTTTAGATCAATTTTTACATGGTGATCAGAAAAAATTAGAAGGTGTTATCAATATT600
CAAAAACGTTTAAAAGAAGTTCAAACAGCTCTTAATCAAGCCCATGGGGAAAGTAGTCCA66
0
GCTCATAAAGAGTTATTAGAAAAAGTAAAAAATTTAAAAACAACATTAGAAAGGACTATT720
AAAGCTGAACAAGATTTAGAGAAAAAAGTAGAATATAGTTTTCTATTAGGACCATTGTTA780
GGATTTGTTGTTTATGAAATTCTTGAAAATACTGCTGTTC AGCATATAAAAAATCAAATT840
GATGAGATAAAGAAACAATTAGATTCTGCTCAGCATGATTTGGATAGAGATGTTAAAATT900

ATAGGAATGTTAAATAGTATTAATACAGATATTGATAATTTATATAGTCAAGGACAAGAA960
GCAATTAAAGTTTT
CCAAAAGTTACAAGGTATTTGGGCTACTATTGGAGCTCAAATAGAA1020
AATCTTAGAACAACGTCGTTACAAGAAGTTCAAGATTCTGATGATGCTGATGAGATACAA108

ATTGAACTTGAGGACGCTTCTGATGCTTGGTTAGTTGTGGCTCAAGAAGCTCGTGATT
TT1140
ACACTAAATGCTTATTCAACTAATAGTAGACAAAATTTACCGATTAATGTTATATCAGAT1200
TCATGTAATTGTTCAACAACAAATATGACATCAAATCAATACAGTAATCCAACAACAAAT1260

ATGACATCAAATCAATATATGATTTCACATGA ATATACAAGTTTACCAAATAATTTTATG1320
TTATCAAGAAATAGTAATTTAGAATATAAATGTCCTGAAAATAATTTTATGATATATTGG1380
TATAATAATTCGGATTGGTATAATAATTCGGATTGGTATAATAAT1425 (2) INFORMATION
FOR SEQ ID
NO:2: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 475 amino acids (B) TYPE:
amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE:
protein (iii) HYPOTHETICAL: YES (iv) ANTI-SENSE: NO (vi) ORIGINAL SOURCE:
(A) ORGANISM: BACILLUS THURINGIENSIS (C) INDIVIDUAL ISOLATE: **PS86A1** (ix)
FEATURE: (A) NAME/KEY: Protein (B) LOCATION: 1..475 (xi) SEQUENCE
DESCRIPTION: SEQ ID NO:2: MetIleIleAspSerLysThrThrLeuProArgHisSerLeuIleHis
151015 T hrIleLysLeuAsnSerAsnLysLysTyrGlyProGlyAspMetThr 202530
AsnGlyAsnGlnPheIleIleSerLysGlnGluTrpAlaThrIleGly 354045
AlaTyrIleGlnThrGlyLeuGlyLeuProValAsnGluGlnGlnLeu 505560 ArgThrHis
ValAsnLeuSerGlnAspIleSerIleProSerAspPhe 65707580
SerGlnLeuTyrAspValTyrCysSerAspLysThrSerAlaGluTrp 859095
TrpAsnLysAsnLeuTyrProLeuIleIleLysSerAlaAsnAspIle 100105110
AlaSerTyrGlyPheLysValAlaGlyAspProSerIleLysLysAsp 115120125
GlyTyrPheLysLysLeuGlnAspGluLeuAspAsnIleValAspAsn MF,300 130135140
AsnSerAspAspAspAlaIleAlaLysAlaIleLysAspPheLysAla 145150155160
ArgCysGlyIleLeuIleLysGluAlaLysGlnTyrGluGluAlaAla 165170175
LysAsnIleValThrSerLeuAspGlnPheLeuHisGlyAsp GlnLys 180185190
LysLeuGluGlyValIleAsnIleGlnLysArgLeuLysGluValGln 19520020 5
ThrAlaLeuAsnGlnAlaHisGlyGluSerSerProAlaHisLysGlu 210215220
LeuLeuGluLysValLysAsnLeuLysThrThrLeuGluArgThrIle 225230235240
LysAlaGluGlnAspLeuGluLysLysValGluTyrSerPheLeuLeu 245250 255
GlyProLeuLeuGlyPheValValTyrGluIleLeuGluAsnThrAla 260265270
ValGlnHisIleLysAsnGlnIleAspGluIleLy sLysGlnLeuAsp 275280285
SerAlaGlnHisAspLeuAspArgAspValLysIleIleGlyMetLeu 290295 300
AsnSerIleAsnThrAspIleAspAsnLeuTyrSerGlnGlyGlnGlu 305310315320
AlaIleLysValPheGlnLysLeuGlnGlyIleT rpAlaThrIleGly 325330335
AlaGlnIleGluAsnLeuArgThrThrSerLeuGlnGluValGlnAsp 340345 350
SerAspAspAlaAspGluIleGlnIleGluLeuGluAspAlaSerAsp 355360365
AlaTrpLeuValValAlaGlnGluAlaArg AspPheThrLeuAsnAla 370375380
TyrSerThrAsnSerArgGlnAsnLeuProIleAsnValIleSerAsp 38539039 5400

SerCysAsnCysSerThrThrAsnMetThrSerAsnGlnTyrSerAsn 405410415
ProThrThrAsnMetThrSerAsn GlnTyrMetIleSerHisGluTyr 420425430
ThrSerLeuProAsnAsnPheMetLeuSerArgAsnSerAsnLeuGlu 435 440445
TyrLysCysProGluAsnAsnPheMetIleTyrTrpTyrAsnAsnSer 450455460
AspTrpTyrAsnAsnSerAspTrpTyrAs nAsn 465470475 (2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 10 amino acids (B) TYPE: amino acid
(C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3: X aaAspPheXaaGlnLeuTyrXaaValTyr 1510
(2) INFORMATION FOR SEQ ID NO:4: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7
amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY:
linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
XaaGluLeuLeuXaaLysVal 15 (2) INFORMATION FOR SEQ ID NO:5: (i) SEQUENCE
CHARACTERISTICS: (A) LENGTH: 12 amino acids (B) TYPE: amino acid (C)
STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi)
SEQUENCE DESCRIPTION: SEQ ID NO:5: LeuGlyProLeuLeuGlyPheValValTyrGlulle 1510
(2) INFORMATION FOR SEQ ID NO:6: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 9
amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY:
linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
AspArgAspValLysIleXaaGlyMet 15 (2) INFORMATION FOR SEQ ID NO:7: (i) SEQUENCE
CHARACTERISTICS: (A) LENGTH: 8 amino acids (B) TYPE: amino acid (C)
STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi)
SEQUENCE DESCRIPTION: SEQ ID NO:7: XaaXaaLysXaaAlaAsnAspIle 15 (2)
INFORMATION FOR SEQ ID NO:8: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20
amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE:
peptide (vi) ORIGINAL SOURCE: (A) ORGANISM: Bacillus thuringiensis (B)
STRAIN: **PS86A1** (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
MetIleIleAspSerLysThrThrLeuProArgHisSerLeuIleHis 1510 15 ThrIleLysLeu 20
(2) INFORMATION FOR SEQ ID NO:9: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 53
base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY:
linear (ii) MOLECULE TYPE: DNA (genomic) (vi) ORIGINAL SOURCE: (A) ORGANISM:
Bacillus thuringiensis (B) STRAIN: **PS86A1** (xi) SEQUENCE DESCRIPTION: SEQ ID
NO:9: ATGATTGATTCTAAAACAACATTACCAAGACATTCWTTAATWCATACWATWAA53
(2) INFORMATION
FOR SEQ ID NO:10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28 bases (B)
TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii)
MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TGATTTTWMTCAATTATATRAKGTTTAT28 (2) INFORMATION FOR SEQ ID NO:11: (i)
SEQUENCE
CHARACTERISTICS: (A) LENGTH: 20 bases (B) TYPE: nucleic acid (C)
STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11: AAGAGTTAYTARARAAAGTA20 (2)
INFORMATION FOR SEQ ID NO:12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35
bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
TTAGGACCATTRYTWGGATTTGTTGTWTATGAAAT35 (2) INFORMATION FOR SEQ ID
NO:13: (i)
SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 bases (B) TYPE: nucleic acid (C)
STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13: GAYAGAGATGTWAAAATYWTAGGAATG27
(2)
INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23
bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TTMTTAAAWCWGCTAATGATATT23 (2) INFORMATION FOR SEQ ID NO:15: (i) SEQUENCE
CHARACTERISTICS: (A) LENGTH: 401 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

US-PAT-NO: **5424410**

DOCUMENT-IDENTIFIER: US 5424410 A
See image for Certificate of Correction

TITLE: Bacillus thuringiensis isolates for controlling acarides

DATE-ISSUED: June 13, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Payne; Jewel M.	San Diego	CA	N/A	N/A
Cannon; Raymond J. C.	Kent	N/A	N/A	GB3
Bagley; Angela L.	Kent	N/A	N/A	GB3

APPL-NO: 08/ 147188

DATE FILED: November 3, 1993

PARENT-CASE:

CROSS-REFERENCE TO A RELATED APPLICATION

This is a division, of application Ser. No. 07/867,280 filed Apr. 30, 1992, now U.S. Pat. No. 5,262,158, which is a continuation-in-part of application Ser. No. 07/693,210, filed on Apr. 30, 1991 now abandoned. This is also a continuation-in-part of application Ser. No. 07/768,141, filed on Sep. 30, 1991 now U.S. Pat. No. 5,211,946, which is a continuation-in-part of application Ser. No. 07/759,248, filed on Sep. 13, 1991, now abandoned.

US-CL-CURRENT: 435/235.1, 424/93.4 , 424/93.46 , 424/93.461 , 435/242 , 435/252.3 , 435/252.33 , 435/252.34 , 435/252.5 , 435/252.8 , 435/320.1 , 435/832 , 536/22.1 , 536/23.1 , 536/23.2 , 536/23.7 , 536/23.71

ABSTRACT:

Disclosed and claimed are Bacillus thuringiensis isolates designated B.t. PS50C, B.t. **PS86A1,** B.t. PS69D1, B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t.. PS24J, B.t. PS94R3, B.t. PS17, B.t. PS62B1 and B.t. PS74G1 which are active against acaride pests. Thus, these isolates, or mutants thereof, can be used to control such pests. Further, genes encoding novel .delta.-endotoxins can be removed from these isolates and transferred to other host microbes, or plants. Expression of the .delta.-endotoxins in microbe hosts results in the control of acaride pests, whereas transformed plants become resistant to acaride pests.

2 Claims, 3 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 2

---------- KWIC ---------

Abstract Text - ABTX (1):

Disclosed and claimed are Bacillus thuringiensis isolates designated B.t. PS50C, B.t. **PS86A1,** B.t. PS69D1, B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t.. PS24J, B.t. PS94R3, B.t. PS17, B.t. PS62B1 and B.t. PS74G1 which are active against acaride pests. Thus, these isolates, or mutants thereof, can be used to control such pests. Further, genes encoding novel .delta.-endotoxins can be removed from these isolates and transferred to other host microbes, or plants. Expression of the .delta.-endotoxins in microbe hosts results in the control of acaride pests, whereas transformed plants become resistant to acaride pests.

US Patent No. - PN (1):

5424410

Brief Summary Text - BSTX (14):

More specifically, the subject invention concerns Bacillus thuringiensis isolates designated B.t. PS50C, B.t. **PS86A1,** B.t. PS69D1, B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J, B.t. PS94R3, B.t. PS17, B.t. PS62B1 and B.t. PS74G1.

Detailed Description Text - DETX (30):

SEQ ID NO. 29 is the nucleotide sequence of a gene from **PS86A1**.

Detailed Description Text - DETX (31):

SEQ ID NO. 30 is the amino acid sequence of the protein expressed by the gene from **PS86A1**.

Detailed Description Text - DETX (46):

It should be apparent to a person skilled in this art that genes coding for acaride-active toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described below. These genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases

or endonucleases according to standard procedures. For example, enzymes such as Ba131 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active **fragments of these toxins**.

Detailed Description Text - DETX (47):

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from B.t. isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the acaride-active toxins of the instant invention which occur in nature. For example, antibodies to the acaride-active toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the acaride-active toxins using procedures which are well known in the art. These antibodies can then be used to specifically identify equivalent toxins with the characteristic acaricidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent **toxins, or fragments of these toxins,** can readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

Detailed Description Paragraph Table - DETL (2):

Strain	Crystal Type	Approx. Mol. Wt. of Proteins (kDa)
B. thuringiensis PS50C	Sphere	135 doublet
B. thuringiensis **PS86A1**	Multiple	45, 58
B. thuringiensis PS69D1	Elongated	34, 48, 145
B. thuringiensis PS72L1	Long rectangle	42, 50
B. thuringiensis PS75J1	Amorphic	63, 74, 78, 84
B. thuringiensis PS83E5	Multiple	37, 42
B. thuringiensis PS24J	Long	51, 48, 43
B. thuringiensis PS94R3	Long	50, 43, 42
B. thuringiensis PS45B1	Multiple	150, 135, 35
B. thuringiensis PS17	Long	155, 145, 128
B. thuringiensis PS62B1	Attached multiple	35
B. thuringiensis PS74G1	Amorphic	148, 112, 104, 61

Detailed Description Paragraph Table - DETL (3):

Culture	Accession No.	Deposit Date
B.t. PS50C	NRRL B-18746	January 9, 1991
B.t. **PS86A1**	NRRL B-18400	August 16, 1988
B.t. PS69D1	NRRL B-18247	July 28, 1987
B.t. PS72L1	NRRL B-18780	March 7, 1991
B.t. PS75J1	NRRL B-18781	March 7, 1991
B.t. PS83E5	NRRL B-18782	March 7, 1991
B.t. PS45B1	NRRL B-18396	August 16, 1988
B.t. PS24J	NRRL B-18881	August 30, 1991
B.t. PS94R3	NRRL B-18882	August 30, 1991
B.t. PS17	NRRL B-18243	July 28, 1987
B.t. PS62B1	NRRL B-18398	August 16, 1988
B.t. PS74G1	NRRL B-18397	August 16, 1988
E. coli NM522(pNffC 2321)	NRRL B-18770	February 14, 1991
E. coli NM522(pMYC 2317)	NRRL B-18816	April 24, 1991
E. coli NM522(pNffC 1627)	NRRL B-18651	May 11, 1990
E. coli NM522(pMYC 1628)	NRRL B-18652	May 11, 1990
E. coli NM522(pMYC 1638)	NRRL B-18751	January 11, 1991
E. coli NM522(pMYC 1638)	NRRL B-18769	February 14, 1991

Detailed Description Paragraph Table - DETL (5):

TABLE 2 _____ Toxicity of Bacillus thuringiensis isolates to the two spotted spider mite, Tetranychus urticae. Mortality was determined after 7 days of treatment. Percent Isolate Mortality _____ B.t. PS50C 63 B.t. **PS86A1** 85 B.t. PS69D1 77 B.t. PS72L1 85 B.t. PS75J1 85 B.t. PS83E5 70 B.t. PS45B1 82 B.t. PS24J 90 B.t. PS94R3 97 B.t. PS17 >90 B.t. PS62B1 >90 B.t. PS74G1 >90 Control 10 _____

Detailed Description Paragraph Table - DETL (10):

340345350 ThrSerLeuLysGluIleGluGluGluAsnAspAspAspAlaLeuTyr 355360 365 IleGluLeuGlyAspAlaAlaGlyGlnTrpLysGluIleAlaGluGlu 370375380 AlaGlnSerPheValLeuAsnAlaTyrThrPro 385390 395 (2) INFORMATION FOR SEQ ID NO:10: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 22 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
AGARTRKWTWAATGGWGCKMAW
22 (2) INFORMATION FOR SEQ ID NO:11: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 8 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11: ProThrPheAspProAspLeuTyr 15 (2) INFORMATION FOR SEQ ID NO:12: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 14 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
AlaIleLeuAsnGluLeuTyrProSerValProTyrAsnVal 1 510 (2) INFORMATION FOR SEQ ID NO:13: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 14 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13: AlaIleLeuAsnGluLeuTyrProS erValProTyrAsnVal 1510 (2) INFORMATION FOR SEQ ID NO:14: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
MetIleIleAspSerLysThrThrLeuProArgHisSerLeuIleAsn 151015 Thr (2) INFORMATION FOR SEQ ID NO:15: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 24 amino acids (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
MetIleLeuGlyAsnGlyLysThrLeuProLysHisIleArgLeuAla 1510 15
HisIlePheAlaThrGlnAsnSer 20 (2) INFORMATION FOR SEQ ID NO:16: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16: GCAATTTTAAATGAATTATATCC23 (2) INFORMATION FOR SEQ ID NO:18: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 38 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:
AAACATATTAGATTAGCACATATTTTTGCAACACAAAA38 (2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA

(synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19: CAAYTACAAGCWCAACC17 (2) INFORMATION FOR SEQ ID NO:20: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO;20: AGGAACAAAYTCAAKWCGRTCTA23 (2) INFORMATION FOR SEQ ID NO:21: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21: TGGAATAAATTCAATTYKRTCWA23 (2) INFORMATION FOR SEQ ID NO:22: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 28 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22: TGATTTTWMTCAATTATATRAKGTTTAT28 (2) INFORMATION FOR SEQ ID NO:23: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 20 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23: AAGAGTTAYTARARAAAGTA20 (2) INFORMATION FOR SEQ ID NO:24: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 35 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24: TTAGGACCATTRYTWGGATTTGTTGTWTATGAAAT35 (2) INFORMATION FOR SEQ ID NO:25: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25: GAYAGAGATGTWAAAATYWTAGGAATG27 (2) INFORMATION FOR SEQ ID NO:26: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 23 bases (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (synthetic) (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26: TTMTTAAAWCWGCTAATGATATT23 (2) INFORMATION FOR SEQ ID NO:27: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 1425 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear (ii) MOLECULE TYPE: DNA (genomic) (iii) HYPOTHETICAL: NO (iv) ANTI-SENSE: NO (vi) ORIGINAL SOURCE: (A) ORGANISM: BACILLUS THURINGIENSIS (C) INDIVIDUAL ISOLATE: **PS86A1** (vii) IMMEDIATE SOURCE: (B) CLONE: E. coli NM522(pMYC1638) NRRL B-18751 (ix) FEATURE: (A) NAME/KEY: mat peptide (B) LOCATION: 1..I425 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27: ATGATTATTGATAGT

AAAACGACTTTACCTAGACATTCACTTATTCATACAATTAAATTA60

AATTCTAATAAGAAATATGGTCCTGGTGATATGACTAATGGAAATCAATTTATTATTTCA120

AAACAAGAATGGGCTACGATTGGAGCATATATTCAGACTGGATTAGGTTTACCAGTAAAT 180

GAACAACAATTAAGAACACATGTTAATTTAAGTCAGGATATATCAATACCTAGTGATTTT240

TCTCAATTATATGATGTTTATTGTTCTGATAAAACTTCAGCAGAATGGTGGAATAAAAAT300

TTATATCCTTTAATTATTAAATCTGCTAATGATATTGCTTCA TATGGTTTTAAAGTTGCT360

GGTGATCCTTCTATTAAGAAAGATGGATATTTTAAAAAATTGCAAGATGAATTAGATAAT420

ATTGTTGATAATAATTCCGATGATGATGCAATAGCTAAAGCTATTAAAGATTTTAAAGCG480

CGATGTGGTATTTTAATTAAAG AAGCTAAACAATATGAAGAAGCTGCAAAAAATATTGTA540

ACATCTTTAGATCAATTTTTACATGGTGATCAGAAAAAATTAGAAGGTGTTATCAATATT600

CAAAAACGTTTAAAAGAAGTTCAAACAGCTCTTAATCAAGCCCATGGGGAAAGTAGTCCA66 GC

TCATAAAGAGTTATTAGAAAAAGTAAAAAATTTAAAAACAACATTAGAAAGGACTATT720

AAAGCTGAACAAGATTTAGAGAAAAAAGTAGAATATAGTTTTCTATTAGGACCATTGTTA780
GGATTTGTTGTTTATGAAATTCTTGAAAATACTGCTGTTCAGCATATAAA AAATCAAATT840
GATGAGATAAAGAAACAATTAGATTCTGCTCAGCATGATTTGGATAGAGATGTTAAAATT900

ATAGGAATGTTAAATAGTATTAATACAGATATTGATAATTTATATAGTCAAGGACAAGAA960
GCAATTAAAGTTTTCCAAAAGTTACAAGGT
ATTTGGGCTACTATTGGAGCTCAAATAGAA1020
AATCTTAGAACAACGTCGTTACAAGAAGTTCAAGATTCTGATGATGCTGATGAGATACAA108

ATTGAACTTGAGGACGCTTCTGATGCTTGGTTAGTTGTGGCTCAAGAAGCTCGTGATTTT11
ACACTAAATG
CTTATTCAACTAATAGTAGACAAAATTTACCGATTAATGTTATATCAGAT1200
TCATGTAATTGTTCAACAACAAATATGACATCAAATCAATACAGTAATCCAACAACAAAT1260

ATGACATCAAATCAATATATGATTTCACATGAATATACAAGTTTACCAAATAATTTT ATG1320
TTATCAAGAAATAGTAATTTAGAATATAAATGTCCTGAAAATAATTTTATGATATATTGG1380
TATAATAATTCGGATTGGTATAATAATTCGGATTGGTATAATAAT1425 (2) INFORMATION
FOR SEQ ID
NO:28: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 475 amino acids (B) TYPE:
amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE:
protein (iii) HYPOTHETICAL: YES (iv) ANTI-SENSE: NO (vi) ORIGINAL SOURCE:
(A) ORGANISM: BACILLUS THURINGIENSIS (C) INDIVIDUAL ISOLATE: **PS86A1** (vii)
IMMEDIATE SOURCE: (B) CLONE: E. coli NM522(pMYC1638) NRRL B-18751 (ix)
FEATURE: (A) NAME/KEY: Protein (B) LOCATION: 1..475 (xi) SEQUENCE
DESCRIPTION: SEQ ID NO:28: MetIleIleAspSerLysThrThrLeuProArgHisSerLeuIleHis
151015 ThrIleLysLeuAsnSerAsnLysL ysTyrGlyProGlyAspMetThr 202530
AsnGlyAsnGlnPheIleIleSerLysGlnGluTrpAlaThrIleGly 3540 45
AlaTyrIleGlnThrGlyLeuGlyLeuProValAsnGluGlnGlnLeu 505560
ArgThrHisValAsnLeuSerGlnAspIleSerIleProSerAspPhe 65 707580
SerGlnLeuTyrAspValTyrCysSerAspLysThrSerAlaGluTrp 859095 TrpAsnLysAsnLeuTyr
ProLeuIleIleLysSerAlaAsnAspIle 100105110
AlaSerTyrGlyPheLysValAlaGlyAspProSerIleLysLysAsp 115120 125
GlyTyrPheLysLysLeuGlnAspGluLeuAspAsnIleValAspAsn 130135140
AsnSerAspAspAspAlaIleAlaLysAlaIleLysAspPheLysAla 1 45150155160
ArgCysGlyIleLeuIleLysGluAlaLysGlnTyrGluGluAlaAla 165170175 LysAsn
IleValThrSerLeuAspGlnPheLeuHisGlyAspGlnLys 180185190
LysLeuGluGlyValIleAsnIleGlnLysArgLeuLysGluValGln 195 200205
ThrAlaLeuAsnGlnAlaHisGlyGluSerSerProAlaHisLysGlu 210215220
LeuLeuGluLysValLysAsnLeuLysThrThrLeuGlu ArgThrIle 225230235240
LysAlaGluGlnAspLeuGluLysLysValGluTyrSerPheLeuLeu 245250 255